

Mestrado em Biodiversidade, Genética e Evolução

Leonese dialects in the Portuguese-Spanish border: a population genetic study through the analysis of X-chromosomal markers



Instituto de Patologia e Imunologia Molecular da Universidade do Porto



Joana Filipa de Oliveira Correia Pinto

Dissertação orientada por: Professora Doutora Maria João Prata
Luís Fernandez, PhD

Agradecimentos

O meu reconhecido agradecimento

À direcção do IPATIMUP e ao Professor António Amorim pela forma como fui recebida e por me terem sido proporcionadas todas as condições para a realização deste trabalho.

À Professora Maria João Prata e Luís Fernandez pela excelente oportunidade de participar neste trabalho, pela constante disponibilidade e valiosa ajuda na elaboração desta tese.

À Vânia por todo o apoio prestado desde o início, pela paciência e boa disposição.

A todo o grupo de Genética Populacional pelo óptimo acolhimento.

A todos os dadores anónimos que se disponibilizaram a participar neste estudo.

Index

Figure Index	iii
Table Index	v
1. Resumo	2
Abstract	5
2. Introduction	
2.1. Population genetics studies in the Iberian Peninsula: a common genetic background with other European regions	8
2.2. The anthropological interest of studying the “Raia”, the Portuguese-Spanish border	10
2.3. X chromosome in Population Genetics	16
2.3.1. Microsatellites (STRs)	19
2.3.2. Insertion/Deletion Polymorphisms (Indels)	21
2.4. Objectives	23
3. Materials and Methods	
3.1. DNA Samples	25
3.2. DNA extraction	25
3.3. X Chromosomal markers	25
3.3.1. Amplification of the X-STRs	25
3.3.2. Amplification of the X-Indels	29
3.3.3. Fragment detection	31
3.3.4. Decaplex amplification	32
3.4. Statistical Analysis	33
3.4.1. Intrapopulation analysis	33
3.4.2. Comparative analysis	34

4. Results and Discussion	38
4.1. Miranda do Douro and Zamora diversity	38
4.1.1. X-STRs	38
4.1.1.1. Hardy-Weinberg Equilibrium	42
4.1.1.2. Genetic distance - F_{ST}	42
4.1.1.3. Linkage Disequilibrium	44
4.1.2. X-Indels	46
4.1.2.1. Hardy-Weinberg Equilibrium	47
4.1.2.2. Genetic distance - F_{ST}	48
4.1.2.3. Linkage Disequilibrium	49
4.1.3. STRs + Indels	50
4.2. Analysis of barriers	53
4.3. Comparative analysis	56
5. Conclusion	60
6. References	63
7. Supplementary Material	vii

Figure Index

Figure 1 - Dialect distribution in the Iberian Peninsula around the 10th century (Astur-Leonese dialect highlighted).

Figure 2 - Geographic distribution of the western (blue tones) and central (purple tones) Astur-Leonese language dialectal variants across the Iberian Peninsula. Geographic location of the Bragança district in its administrative unit context: districts in Portugal and provinces in Spain. Miniature map - The Portuguese (white color) and Spanish (grey color) territorial expanse in the Iberian Peninsula, highlight of its northwestern quadrant.

Figure 3 - Geographic representation of: 1- Bragança district (Miranda do Douro highlighted); 2- Zamora municipality.

Figure 4 - X chromosome mode of inheritance. The X chromosome is displayed in colours; in the maternal transmission, recombination occurs (“Recombination”).

Figure 5 - Ideogram of the X chromosome indicating the location of the 32 selected INDEL markers [from *Pereira et al.* 2012].

Figure 6 - The ideogram of the X-chromosome describes the physical localization of the STR loci which can be analysed with the Investigator Argus X-12 Kit [Extracted from Investigator X-12 Handbook 2010, Qiagen].

Figure 7 - Electropherogram of the control sample DNA XX28 for the 12 X-STRs.

Figure 8 - Electropherogram of the control sample 9947A for the 32 X-Indels.

Figure 9 - Electropherogram of the allelic ladder Argus X-12 analyzed on an ABI PRISM 310 Genetic Analyzer [Adapted from the Investigator Argus X-12 Kit Handbook 2010].

Figure 10 - Multiplex used for the 10 X-STRs, with reference to the interval sizes expected for each marker in base pairs (bp). Also represented are the fluorescent colours attributed to each marker (blue: 6-FAM; green: VIC; yellow: NED)

Figure 11 - Detail of the electropherogram of one of the samples presenting ambiguous allele for the 12 X-STRs kit.

Figure 12 - Detail of the electropherogram of the sample showed in figure 11 for the Decaplex kit (10 X-STRs).

Figure 13 - Detail of electropherogram of the triallelic sample (12 X-STRs).

Figure 14 - Map of X-chromosomal markers in close LD based on their genetic positions according to Rutgers map.

Figure 15 - Maps displaying the considered barriers: a) political, b) geographical and c) linguistic.

Figure 16 - Three main barriers detected in the Barrier analysis. The thickness of each edge of the barrier is proportional to the number of times it was included in the computed barriers (numbers on edges).

Figure 17 - Plot of the Multidimensional Scaling analysis of 8 X-STRs (abbreviations in Materials and Methods section).

Figure 18 - Plot of the Multidimensional Scaling analysis of the studied populations for 12 X-STRs, considering markers in LD.

Figure 19 - Plot of the Multidimensional Scaling analysis of 32 X-Indels, considering markers in LD.

Table Index

Table 1 - Characteristics of the 12 X-STR markers used [Partly extracted from Investigator Argus X-12 Handbook 2010].

Table 2 - Set up of the master mix used in the amplification with the Investigator Argus X-12 kit.

Table 3 - Thermocycling conditions used for the DNA amplification with the Investigator Argus X-12 kit.

Table 4 - Characteristics of the 32 X chromosome Indels used [Extracted from Pereira et al. (2011)].

Table 5 - Set up of the mix used in the amplification of 32 X-Indels with the Qiagen Multiplex PCR kit.

Table 6 - Thermocycling conditions used for the 32 X-Indel multiplex kit.

Table 7 - Set up used in the capillary electrophoresis of the amplification products with the Investigator Argus X-12 Kit and 32 X-indel kit.

Table 8 - Samples used in the comparative analysis of X-STRs and X-Indels.

Table 9 - Allele frequencies and heterozygosity for 12 X-STRs in Miranda and Zamora.

Table 10 - Results of HWE test for 12 X-STRs, in a sample of 66 females from Miranda and 86 females from Zamora.

Table 11 - Genetic distances (F_{ST}) between six regions of Zamora and Miranda for 12 X-STRs.

Table 12 - Genetic distances (F_{ST}) between the Zamora regions, Miranda, Portugal and Galicia for 8 X-STRs.

Table 13 - Genetic distances (F_{ST}) considering LD between the Zamora regions and Miranda for the 12 X-STR markers.

Table 14 - Allelic frequencies and heterozygosity for 32 X-Indels. Only the frequencies of the short alleles are presented.

Table 15 - Results of HWE test to the 32 X-Indels, in a sample of 66 females from Miranda and 86 females from Zamora.

Table 16 - Genetic distances (F_{ST}) between six regions of Zamora and Miranda for 32 X-Indels.

Table 17 - Genetic distances (F_{ST}) between the regions of Zamora, Miranda, Portugal, Somalia, Angola-Mozambique (Ang-Moz), Macau and Iraq for 32 X-Indels.

Table 18 - Genetic distances (F_{ST}) considering LD between the Zamora regions and Miranda for the 32 X-Indel markers.

Table 19 - Average gene diversity (H) and mean number of pairwise differences (M and the corresponding standard deviations, $M \pm s.d.$) in Miranda do Douro and Zamora

populations. Corrected values for each set of markers are also presented after detection of linkage disequilibrium in several pairs.

Table 20 - Results obtained from the AMOVA analysis, regarding 8 X-STRs and 32 X-Indels.

Table S1 - Genetic profiles obtained with the amplification of the 12 X-STRs.

Table S2 - Genetic profiles obtained with the amplification of the 32 X-Indels.

Table S3 - X-chromosomal markers analysed in this study and their physical and genetic locations.

Table S4 - F_{ST} obtained for 8 X-STRs used in the MDS analysis.

1. Resumo

A Península Ibérica, situada no Sudoeste da Europa, possui uma história recente complexa que a torna um tema de estudo interessante em termos de diversidade genética, bem como para pesquisas histórico-linguísticas. Alguns estudos recentes de SNPs autossómicos a grande escala mostraram que a estrutura genética da população europeia está intimamente relacionada com a sua geografia. Além disso, um estudo de marcadores do cromossoma X apontou para uma correlação de limites linguísticos em regiões europeias que exibem nítidas mudanças genéticas.

A instituição da fronteira entre Portugal e Espanha em 1267 criou uma nova barreira entre as populações, no entanto aldeias situadas em toda a região de fronteira detêm aspectos culturais e históricos compartilhados por ambos os países, como consequência de uma longa história em comum, da fácil migração de pessoas entre eles e, até recentemente, a actividade de contrabando tradicional. Um grande sinal desta troca recíproca entre os dois países é a sua história linguística. A continuidade de um antigo dialecto leonês nas regiões Norte e Ocidental da Península Ibérica tem sido relatado em ambos os países. Em Portugal, o idioma que foi preservado é conhecido como o Mirandês, que é considerado uma variante do dialecto leonês e representa um isolado linguístico, apesar da sua pequena extensão. Na realidade, as pessoas de Miranda do Douro são muitas vezes bi ou trilingues, falando o Português - a principal língua em Portugal, o castelhano - a língua dominante do país vizinho, e o Mirandês.

A presença de características linguísticas comuns em Miranda e outras regiões espanholas vizinhas, como Zamora, tem atraído muita atenção. No entanto, muitas incertezas ainda persistem sobre a origem e manutenção deste isolado linguístico. Duas hipóteses principais foram colocadas para explicar a consolidação deste dialecto na região: uma admite uma origem indígena, enquanto a outra o considera resultado de diversas iniciativas de colonização realizadas pelo Reino de León. Apesar do grau de incerteza, a persistência do dialecto leonês na área pode ser o resultado do isolamento geográfico intrínseco a Miranda, particularmente no passado quando as vias de comunicação eram muito debilitadas, tornando difícil o acesso à região.

Neste estudo, a caracterização genética de populações de Miranda do Douro e Zamora foi realizada através da análise de X-STRs e X-indels, no âmbito de um projecto de populacional genético global, que também inclui a análise de diversidade do mtDNA e do cromossoma Y, visando a obter uma melhor compreensão da ligação entre os padrões de diversidade genética, geografia, e os dialectos leoneses falados em ambas as regiões.

Depois de analisar os dois tipos de marcadores do cromossoma X, tanto Miranda do Douro e Zamora partilham razoavelmente os padrões de diversidade normalmente encontrados em populações da Península Ibérica.

A análise do contexto microgeográfico evidencia a ligação entre Miranda e Zamora, apesar de uma subestrutura genética significativa não ter sido detectada.

A fronteira política parecia ser uma forte barreira impedindo o fluxo genético entre as populações de fronteira, chegando mesmo a isolar todas as áreas espanholas das portuguesas, como havia sido relatado anteriormente.

Tanto Miranda do Douro e Zamora se apresentaram altamente diversificadas para todos os marcadores do cromossoma X. Miranda tendeu a apresentar níveis de diversidade ligeiramente mais baixos, mas mesmo assim sinais importantes de deriva genética não foram observados no seu conjunto de genes, o que leva a presumir que o efeito de isolamento e o pequeno tamanho populacional provavelmente foram neutralizados por outros factores demográficos.

A ausência de heterogeneidade em Miranda comparativamente a diversidade a nível do cromossoma X que caracteriza a população portuguesa em geral leva a que Miranda não necessite de ser considerada diferenciadamente no campo da genética forense.

No geral, nossos resultados só forneceram fracas indicações sobre as relações entre Miranda e as regiões vizinhas de Zamora. Provavelmente porque as respostas para as questões abordadas neste estudo dependem de características subtis que diferenciam os perfis genéticos das populações podendo assim escapar à resolução fornecida pela análise deste marcadores do cromossoma X.

1. Abstract

The Iberian Peninsula, lying in the South-Westernmost region of Europe, possesses a complex recent history which brands it as interesting case study for genetic diversity as well as for historical-linguistic researches. Some recent studies of autosomal SNPs across large-scale samples have shown that the genetic structure of the European population correlates closely with geography. Also, a study of X-chromosomal markers sustained that European regions displaying sharp genetic changes usually tend to correlate to linguistic limits.

The establishment of the Portuguese and Spanish border in 1267 created a new barrier between populations, however villages situated in the entire border region detain cultural and historical aspects shared by both countries, as a consequence of a long shared history, the ease migration of people between them and, up to recently, the traditional smuggling activity. A great signal of this reciprocal exchange between the two countries is their linguistic history. The survival of an old Leonese dialect in the North and Occidental regions of the Iberian Peninsula has been described as encompassing both countries. In Portugal, the language that is preserved is known as the “Mirandês”, which is considered a variant of the Leonese dialect and represents a linguistic isolate in Portugal, although of extremely small range. As a matter of fact, people from Miranda do Douro are often bi or trilingual, speaking the Portuguese – the major language in Portugal, the Castilian – the dominant language in the neighbour Spanish region, and the “Mirandês”.

The presence of common linguistic characteristics in Miranda and other neighbour Spanish regions, such as Zamora, has attracted much attention. However, many uncertainties still persist about the origin and maintenance of such linguistic isolate. Two main hypotheses have been hold to explain the consolidation of this dialect in the region: one admits an indigenous origin, while the other considers it a result of several settlement initiatives carried out by the Kingdom of Leon. Despite the level of uncertainty, the persistence of the Leonese dialect in the area may be the result of Miranda’s intrinsic geographic isolation, especially in the past when communication routes were very deficient, rendering difficult access to the region.

In this study, the genetic characterization of the populations of Miranda do Douro and Zamora was carried out through the analysis of X-STRs and X-INDELs, in the scope of a comprehensive population genetic project, which also includes the analysis of mtDNA and Y chromosome diversity, aimed at obtaining a better understanding on the connection between patterns of genetic diversity, geography, and the Leonese dialects spoken in both regions.

After analyzing both kinds of X-chromosomal markers, both Miranda do Douro and Zamora fell reasonably in the diversity patterns usually found in Iberian Peninsula populations.

The dissection of the microgeographical context illustrated the connection between Miranda and Zamora, even though no statistically significant genetic substructure was detected.

The political frontier appeared to be a strong barrier hampering gene flow between border populations, actually isolating all the Spanish areas from the Portuguese areas, as it had been previously reported.

Both Miranda do Douro and Zamora were highly diverse for all X-chromosomal markers. Miranda tended to present slightly lower levels of diversity, but even so no major signs of genetic drift were observed in its gene pool leading to presume that the effect of isolation and small population size were likely neutralized by other demographic factors.

The absence of heterogeneity in Miranda comparatively to X-chromosomal diversity that characterises the general population from Portugal implies that Miranda does not need to be differentially considered in the field of Forensic Genetics.

Overall, our findings only provided faint hints on the relationships between Miranda and the neighbour regions from Zamora. Probably because the answers to the questions addressed in this study rely in subtle features differentiating the genetic profiles of the populations that can escape the resolution provided by the analysis of X-chromosomal markers.

2. Introduction

2.1. Population genetics studies in the Iberian Peninsula: a common genetic background with other European regions

Europe is the most exhaustively studied area in the world concerning the evolution of human population through the use of genetic analysis. Whilst other sources, such as archaeology, help infer the cultural impact of the historical events, these can often clash with the evidences of the demographic impact. Therefore, resorting to the genetic analysis of modern populations can provide a more straightforward tactic in assessing the impact of migrations and invasions in history [1]. Past studies acquired most of their knowledge from what are known as classical genetic markers, such as protein or blood group genetic polymorphisms, which were extensively analysed regarding patterns of geographic variation of allele frequencies. Cavalli-Sforza *et al.* [2] recognized the primarily interpretation of the genetic variation in Europe as being mainly modelled by the demographic impact of the Neolithic expansion [3]. But if there is a need for genetic studies to focus on the identification of large-scale variation that might foster the understanding of the influence of prehistoric events in the current day genetic landscape of Europe, it is as well important to address the effects of migrations and invasions in more recent times at a micro-geographical level, considering their major importance in shaping extant patterns of diversity [1].

In this context, Europe is one of the well-known regions in the world to have been subjected to several migratory events, which might have had an important role in the modelling of the diversity patterns observed nowadays [4]. These recent events along with the more ancient population movements can contribute to a misleading understanding of the complex history of the human populations.

After entering the era of the molecular genetic markers, the more comprehensive studies on European genetic diversity have mainly rested upon the investigation of mitochondrial and Y-chromosome DNA, which despite having the advantage of allowing for easily inference of haplotypes able to afford rather robust interpretations, are very prone to genetic drift effects besides being only representative of one part of the genetic history, mediated by female or male, respectively [5].

More recently, high-throughput genotyping of autosomal SNP across large-scale samples of Europeans reinforced previous studies indicating that the European population lacked sharp discontinuities, but despite the low average levels of genetic differentiation among Europeans, the genetic structure of the European population correlates closely with geography [6,7].

The analysis of European diversity through X-chromosomal markers has been seldom investigated, but in 2004, Xiao *et al.* [5] using X-haplotypes showed that the demographic history of Europe has been influenced, not only by settlements from the Near East, but also by other major population movements, such as expansions from Asia and a more recent gene flow within Europe and the Middle East.

Previous studies in Europe had indicated that regions displaying the sharpest genetic change usually tend to correlate to linguistic limits [8,9]. Furthermore, numerous investigations have been performed regarding European isolated populations and their genetic composition, in order to refine the knowledge on human diversity and history, and often to evaluate whether specific population isolates could be used in disease mapping studies.

The Iberian Peninsula, lying in the South-Westernmost region of Europe, possesses a complex recent history, including over the last two millennia, which brands it as an interesting case study for genetic diversity as well as for historical-linguistic matters [1,10]. Iberia is quite well integrated in the context of the European genetic diversity, and as a whole is characterised by a relative genetic homogeneity. Yet, at a more restricted geographic scale, some signs of genetic structure have been confirmed several times, with the Basques representing one of the European populations that have drawn stronger attention of geneticists and that often showed heterogeneity. Their differentiation from neighbouring populations, as revealed by differences in gene frequencies at several markers, has been explained as the result of specific events of genetic drift in the course of their history [3].

Also in the Mediterranean region some signs of genetic specificity can be found, reflecting the fact that the Iberian Peninsula and North-Western Africa are not only in close geographical proximity, but are also linked by historical events involving population movements. Studies regarding mtDNA show that these movements affected the genetic composition of the Iberian Peninsula, leaving imprints clearly distinguishable from those left by other European influences. Despite the difficulty in obtaining a faithful estimate of the demographic contribution that the North-Western African population provided to Iberia, history confirms its presence. For instance, Berber troops, in A.D. 711, under Arab leadership [11] conquered the Peninsula, beginning a period of 8 centuries during which the Iberian Peninsula was divided into the Christian kingdoms to the north and the Islamic kingdoms in the south [12,13].

The information gathered from mtDNA confirms not only the presence of North African but also of sub-Saharan lineages in the Peninsula [14,15].

Most of the sub-Saharan lineages that are currently found in Iberian populations can be related to the well-known history of the massive transatlantic African slave trade that lasted from 16th until the 19th century. The slaves were mainly captured along the West African Coast, being then regularly transported to Cape Verde and other islands, which were used from the beginning of the trade as platforms of connection between the African continent and Europe, America and even India [15,16,17].

Signs of the major population influences in Europe have been coherently captured in different genetic studies. However, further analyses on yet unstudied Iberian isolated populations are needed to better understand the relative influence of pre-historic and historic settlers and migrants, from different geographic backgrounds, on the generation of microgeographic population structure in the Iberian context [10].

2.2. The anthropological interest of studying the “Raia”, the Portuguese-Spanish border

Studies using archaeological and historical data available for the North-Western extreme of the Iberian plateau expose a temporal continuum for human activity since the first human settlements, dating from the mid-Pleistocene period to the present [15].

The Portuguese and Spanish border was established in 1267, with an extension of over 1000km, making it one of the oldest and largest frontiers in Europe. Villages situated in the entire border region detain cultural and historical aspects shared by both countries, as a consequence of the ease migration of people between them and, up to recently, the traditional smuggling activity. Moreover, in the last centuries, ethnic and religious minorities, such as *moriscos* and Sephardic Jews, used the border region as a refuge from Christian imposition [1].

A signal of the reciprocal exchange between the two countries is their linguistic history. In the Iberian Peninsula, four initial dialectical forms of the Vulgar Latin were already established in the early Reconquista period initiated soon after the arrival of Muslims into Iberia in 711 BC: Astur-Leonese, Castilian, Navarro-Aragonese and Catalan. From the first group the Gallego differentiated, later originating the Portuguese language.

The Leonese dialect is usually considered to be a derivative of Castilian, when actually, it is significantly different, even considering that both languages derive from common Latin, Leonese was for several centuries the predominant language across Christian Spain, from the frontier with France to Galicia [18].



Figure 1 Dialect distribution in the Iberian Peninsula around the 10th century (Astur-Leonese dialect highlighted).

In the early years of the 20th century, Menéndez Pidal [19] describes the survival of this old dialect in the North and Occidental regions of its domain. The Occidental region refers to, among others, part of Zamora and of Portugal (“...by west of Puebla de Sanabria to the Portuguese border. Enters in Portugal, taking the area of Miranda do Douro...”). The language that is preserved in the Portuguese territory is the “Mirandês”, which is considered a variant of the Leonese dialect and represents a linguistic isolate in Portugal, although of extremely small range. As a matter of fact, people from Miranda do Douro are often bi or trilingual, speaking the Portuguese – the major language in Portugal, the Castilian – the dominant language in the neighbour Spanish region, and the “Mirandês” – considered to be a Leonese dialect that in Portugal is exclusively retained in that region, being spoken by only a few thousand people (10.000-15.000) [20]. The presence of this linguistic characteristic in Miranda has attracted much attention, but many uncertainties still persist about the origin and maintenance of such linguistic isolate.

This language has also been noted in some villages in the nearby municipalities of Vimioso and Mogadouro. Within the region, three variants can be identified: the “Mirandês central”, the “Mirandês raiano”, which is close to the border with Zamora and the “Sendinês”, in the southern region, centered on the village of Sendim [20,21].

As mentioned earlier, the linguistic affiliation of the “Mirandês” has been primarily discussed by Menéndez Pidal and José Leite de Vasconcelos, who have agreed to the deep intimate relation between “Mirandês” and Leonese. Moreover, Vasconcelos states, in 1882, that the “Mirandês” belongs to the Spaniard domain, close to the Leonese, but that the influence of the Portuguese in the dialect is also undeniable, as a result of political forces [22]. So, the “Mirandês” reveals a fundamental Spanish character with a strong influence of the Portuguese phonology. Several other investigators have since then studied the linguistic characteristics of this dialect like Carolina Michaelis de Vasconcelos (1913) [23], Maria José Moura Santos (1967) [24], Leif Sletsjøe (1967) [25], António Mourinho (1987) [26] and more recently Luísa Segura da Cruz, João Saramago e Gabriela Vitorino (1994) [27] and Manuela Barros Ferreira (1999) [28]. Regarding the origins of the “Mirandês” different interpretations have been defended, causing in certain cases some controversy.

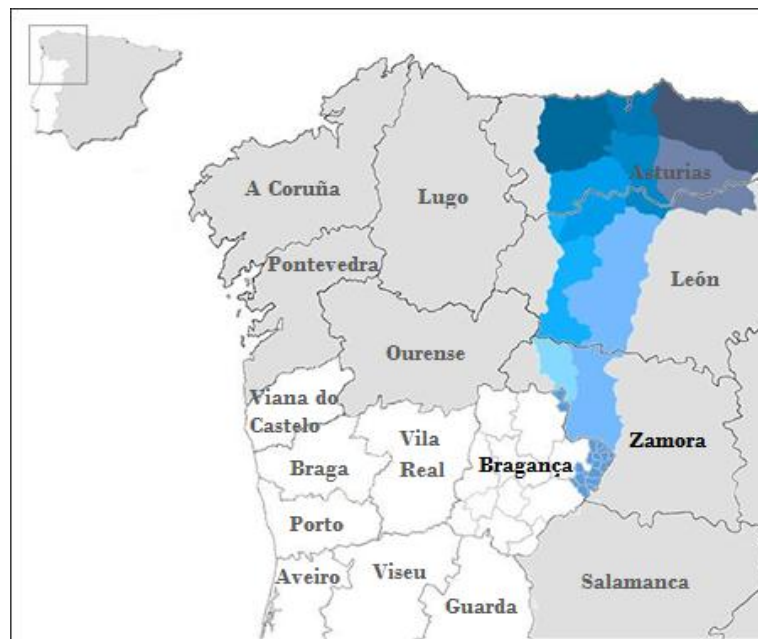


Figure 2 Geographic distribution of the western (blue tones) and central (purple tones) Astur-Leonese language dialectal variants across the Iberian Peninsula. Geographic location of the Bagança district in its administrative unit context: districts in Portugal and provinces in Spain.
Miniature map - The Portuguese (white color) and Spanish (grey color) territorial expanse in the Iberian Peninsula, highlight of its northwestern quadrant.

In order to discuss or hypothesize the origins of any language it is important to understand the history of the region concerned.

Prior to the arrival of the Romans, several tribes occupied the Iberian Peninsula, particularly the asturian tribe Zoelas that inhabited Miranda and surrounding territories. This tribe is described as having a marked individuality and an organization that

prevailed well into the 2nd century, with an already high level of acculturation without losing their individuality. This strong identity included their language, which slowly began to be affected by the Latin brought in by the Roman legions. With this, the people from these regions started integrating their own language structures in the Latin language, introducing words, expressions and other elements or changing the Latin words themselves. António Mourinho, in 1980 [29], speaks of a pact between the Romans and the Zoelas, establishing limits to the legal convents of Asturias and Braga: a border crossing Castro de Avelãs near Brangança, with Braga to the west and Astorga to the east. Miranda was disputed by both dioceses, finally becoming part of the Braga diocese only after the Reconquista (discussed later).

Accordingly, from the linguistic point of view, one of the most important historical phases in the Iberian Peninsula was initiated with the arrival of the Romans, around 219 BC, which besides leading to a systematic change in customs and living conditions for the endemic populations, also brought one of their most significant contributions to the peninsula: the introduction of Latin, from which derive all the romance languages nowadays spoken in Iberia [19].

A period that significantly affected the demographic and linguistic history of nowadays cross-border area between Portugal and Spain spanned from the mid 8th century to the 11th century, when land along the Douro River line remained practically uninhabited [15]. Some historians, like Cláudio Sanchez-Albornoz [30] and Herculano de Carvalho [31], claim that, with the arrival of Muslims in 711 BC, the Christians took refuge in the North of the Peninsula, in Galicia and the Asturian hills, creating between the rivers Douro and Tejo a vast desert area that became a defensive corridor separating the Christian and Muslim kingdoms. This situation could have later led to the repopulation of the desert region, probably with people from Leon, who already spoke the Leonese dialect, and such circumstances might help explain why a Leonese variant is currently spoken in the Miranda do Douro area. This repopulation of the desert area between the rivers Douro and Tejo, has been hypothesised to have triggered the beginning of the consolidation of a Leonese dialectic in Miranda, but the explanation has been discarded by some specialists [32], since the actual endemic populations of those supposed deserted regions, are likely to have remained there, as showed by the continuity of certain costumes prior to that time, indicating that there was an adaptation to the life in these regions without the people ever actually leaving. Also, from a linguistic point of view there are features that characterize the current Leonese dialect of the region, “Mirandês”, that are considered to have arisen prior to these times [33].

Along with the Reconquista that began in the 8th century, culminating in 1492 with conquest of the Algarve by Afonso III, the Leonese dialect extended towards south, occupying a part of the territory from the Cantabric Sea to the province of Badajoz, having its major extension in the 8th century. However, in this area there was still the continuous influence of the Castilian, and it is at this point that the Leonese dialect starts losing ground to the influence of Castilian [18,19].

According to historical data regarding the Zamora province, the territories North of the Douro River, presently the Aliste, Benavente, Campos-Pan and Sanabria regions, gained by Northern kingdoms at the end of the 8th century are thought to have been resettled by individuals from two main origins: Mozarabs, from the Southern Muslim kingdoms, and people from the North-Western kingdoms, nowadays recognized as the territories of Asturias, Galicia and Leon. Considering that the Southern areas of the Douro River, now the Sayago and Bajo-Douro regions, were only resettled when fully incorporated into the Astur kingdom in the 11th century, the historical sources have considered three main different origins for the settlers: individuals from the Northern regions of the Zamora province, people from Al-Andalus (mostly Mozarabs) and individuals from Southern France [15].

On the Portuguese side, with the independence of the *Condado Portucalense*, the frontier between kingdoms changed, and the territories of Bragança and Miranda do Douro were included in the Portuguese kingdom. However, Miranda was able to preserve the commercial and cultural ties to the Leon kingdom, mainly to Zamora, which was closest to Miranda than the politico-cultural Portuguese centers more to the south. Around the 13th century, Miranda do Douro received a new contingent of Leonese immigration, due to the discomfort caused by the fusion of the Leonese and Castellan kingdoms. This re-organization served as a reinforcement of the connection between Leonese-speaking regions [33].

Regarding the Zamora regions, it is known that mainly Aliste and Sanabria spoke and still speak to this day a variant of the Leonese dialect, for example, named “Alistano” in the case of the Aliste region.

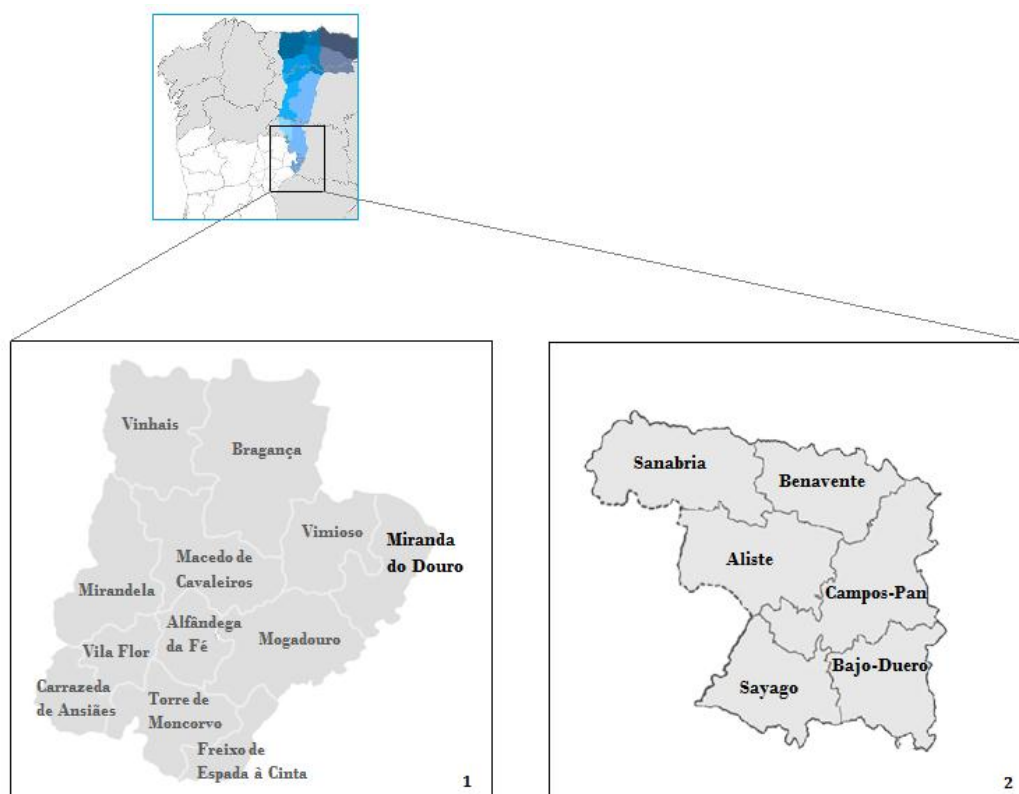


Figure 3 Geographic representation of:
 1- Bragança district (Miranda do Douro highlighted)
 2- Zamora municipality.

The evolution of the Leonese dialect has been affected by the transformations that occurred in both Spanish and Portuguese societies, accelerating its substitution for Castilian or Portuguese languages, especially in the second half of the 20th century. Even the rural areas where the dialect was preserved, have suffered a profound transformation. The massive emigration from the rural areas to the cities, the improvement of the living conditions, allowing more people the access to transportation, the development of telecommunications, has led to lower levels of isolation [33]. All of this should have contributed to the extinction of “Mirandês”, however it is still preserved in Miranda, and it is now considered a minority language [20,21]. This language nowadays survives in conditions similar to the Franco-Provençal language, which are characterized mainly by being languages more spoken than written, more family reserved, more rural than urban. Also, it occupies a certain territory within a country, possesses a long historical tradition, tends to be transmitted from parents to children and has a reduced number of speakers. Still, “Mirandês” has an individuality of its own, quite different from the Portuguese [34].

All of these historical events that the region suffered, such as the belonging to the Astorga diocese, the colonization of people from the Leon Kingdom, the cultural and commercial ties to Zamora and being under the Portuguese influence for centuries, have certainly affected the linguistic evolution of the “Mirandês”, making it a bridge between peoples.

In spite of the contradictory versions revolving around the origins of the Mirandês, two main hypotheses have been thought to explain the consolidation of this dialect in the region: one admits an indigenous origin, while the other considers it a result of several settlement initiatives carried out by the Kingdom of Leon.

Although this level of uncertainty related to the historical events that led to the consolidation of this linguistic differentiation and the persistence of the Leonese dialect in the area is still considerable, Miranda’s intrinsic geographic isolation, particularly in the first half of the century, seems to be the most plausible cause.

In this study, Miranda do Douro is going to be analysed alongside Zamora, since both regions share linguistic traits, due to the Leonese dialects spoken by their people, and also have common historical background, mainly cultural and commercial ties that remained intact over the centuries.

The proximity of the present population to other genetically well-known populations and its peripheral position in European geography makes Miranda, as well as Zamora, interesting for the knowledge of population affinities, history and for refining the geography of genetic variation in Europe.

Hopefully in the future, the genetic information gathered by these multiple studies will become widely available for certain geographic regions, with properly handled data, allowing for a better understanding of our past.

2.3. X chromosome in Population Genetics

The X and Y chromosome are thought to have evolved from an ordinary pair of autosomes, about 300 million years ago, that have accumulated mutations during their evolutionary process [35,36]. Despite their common origin, the sex chromosomes have followed different evolutionary journeys, and actually the proto Y chromosome has lost a considerable amount of genes, including some initially shared with the proto X chromosome, leading to two chromosomes with very distinct structure and functions. Whilst the X chromosome is still able to recombine in females, the Y chromosome only recombines with the X in the pseudoautosomal regions. The human X and Y

chromosomes have two short homologous regions at the telomeres, designated as pseudoautosomal regions 1 and 2, which are autosomal-like and can pair in meiosis [37,38].

Both males and females receive one of their mother's X chromosomes, and females inherit their second X chromosome from their father. Since the father retains his X chromosome from his mother, a human female has one X chromosome from her paternal grandmother and one X chromosome from her mother. So, males have only one X chromosome that isn't subjected to recombination in most of its extension, and therefore the haplotype located in the non-recombining region is transmitted identically to all daughters, unless mutation occurs. Contrary in females, the two X chromosomes undergo meiotic recombination. This specific X chromosome mode of transmission (Figure 4) as well as its recombination patterns, make the study of markers located on this chromosome an important tool in the fields of population and forensic genetics.

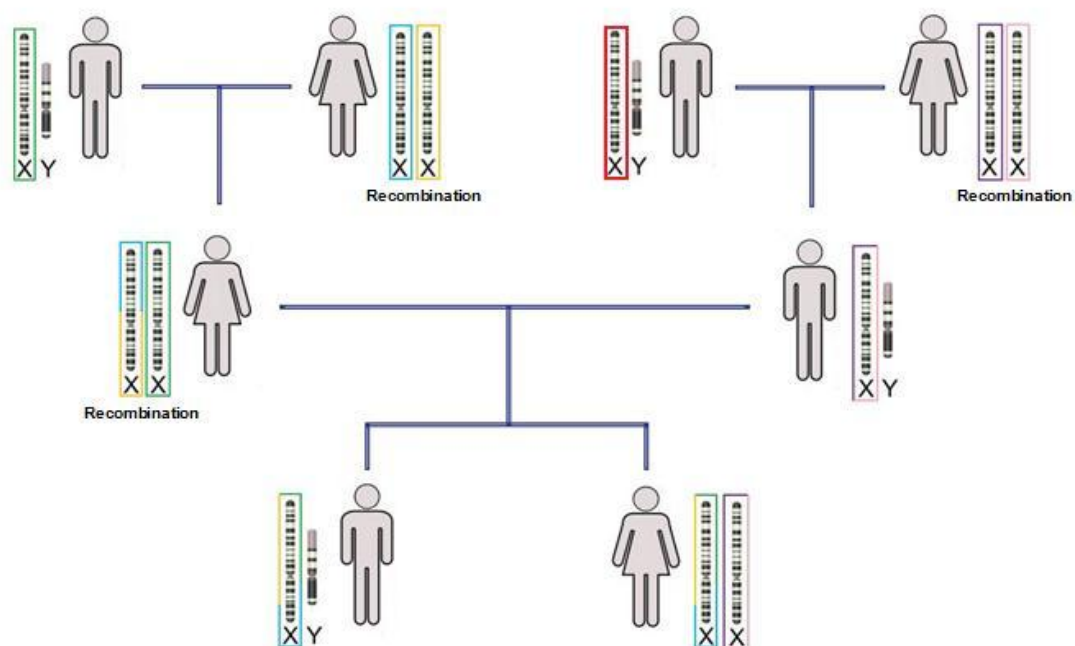


Figure 4 X chromosome mode of inheritance. The X chromosome is displayed in colours; in the maternal transmission, recombination occurs ("Recombination").

The majority of the X chromosome variation studies has been used in the support of large-scale geographical issues, such as the origin of non-African populations, tending to acknowledge the 'Out of Africa' model [39].

Up to now, genetic investigations in the Iberian Peninsula consist mostly of analyses of classical markers, autosomal *Alu* insertion polymorphisms, mitochondrial DNA variation

and Y-chromosomal haplotypes. So, there is a clearly deficit on studies addressing patterns of X-chromosomal diversity, which particularly at a fine geographical scale are in much lower number than human population studies based on Y chromosome and mtDNA variation [39]. In part this can be explained because until recently only a limited number of well-characterized X-linked polymorphisms had been described, for which population data was relatively scarce [40,41], limiting, therefore, the ability to conduct comparative analyses.

However, in more recent years there has been an increasing awareness and interest in the X chromosome, mainly due to its distinctive characteristics and combination of desirable features of other genetic markers. Just as autosomes, the X chromosome suffers recombination and, as mtDNA and Y chromosome, it has a sex-biased mode of inheritance allowing direct haplotyping in males. The fact that the X chromosome is present only in a single copy in male subjects makes it one of its unique features, alongside with exhibiting lower genetic diversity in comparison to autosomal chromosomes [42,43]. The population structure is expected to be more prominent at the X chromosome as a consequence of the reduced effective population size, being three quarters relatively to that of the autosomes, which thereby explains the faster genetic drift of the X in comparison to autosomes. Therefore, populations tend to differ more in their X chromosomes than in their autosomes variation. The linkage disequilibrium is also stronger on the X chromosome, due to only recombining in females, which means only two-thirds of chromosomes recombine in each generation. Consequently, the size of regions with a single genetic history is expected to be larger than in autosomes [44]. These entire characteristics amount to making the X chromosome an ideal source of information, including in haplotyped-based phylogenetic studies often applied in human population genetics and anthropological research [39-54].

Males and females are subject to social practices that may affect evolutionary forces in different way, which can lead to divergent patterns of genetic variation among the autosomes, X chromosome, Y chromosome and the mitochondrial genome [55]. The improvement of sex-specific markers has been influential in better understanding the effects of sex-specific demographic events. The Y chromosome and mtDNA are frequently studied to gather further knowledge on gender-biased demographic processes, in spite the fact that they tend to give limited resolution about ancient demographic occurrences. Complementary to this, the X chromosome and autosomes might afford higher resolution discernment of gender-biased processes due to containing several independent genetic loci whose combined analysis has great potential to shed new lights on previously undocumented events in history [56]. In fact,

numerous demographic histories involving unbalanced migration and breeding patterns of females and males have been revealed by the relative extent of genetic drift between the autosomes and the X chromosome assessed through the comparative analyses of their genetic diversities [57].

Many studies have taken advantage of the differences between males and females concerning several evolutionary factors, such as mutation, recombination, selection, gene flow and genetic drift [58]. Different anthropological investigations focusing on hunter gatherers or other modern populations [59-63] have provided evidence that gender-biased migrations have often occurred during the history of human evolution [63-65].

Recently, for instance, Keinan *et al.* [56], found signs of the occurrence of a period of accelerated genetic drift on the X chromosome around the time of the human dispersal out of Africa. Comparisons of patterns of diversity at the chromosome X and the autosomes, led the authors to conclude that a gender-biased process that reduced the female effective population size, or an episode of natural selection that affected the X chromosome, was associated with the founding of non-African populations.

Also based on the difference in recombination properties between the X chromosome and autosomes, Labuda *et al.* [57] presented a novel approach to assess the breeding ratio in humans, recognising, however, the inherent difficulty in that evaluation, due to the fact of the genetic diversities at the autosomes and the X chromosome being a complex function of both the breeding ratio and the difference in the male and female meiotic mutation rate.

Although the effects of gender-biased mutation and recombination have not been ignored, there's still very little known about the extent to which sex-specific differences in gene flow and genetic drift have shaped patterns of variation at the level of the genome [58].

2.3.1. Microsatellites (STRs)

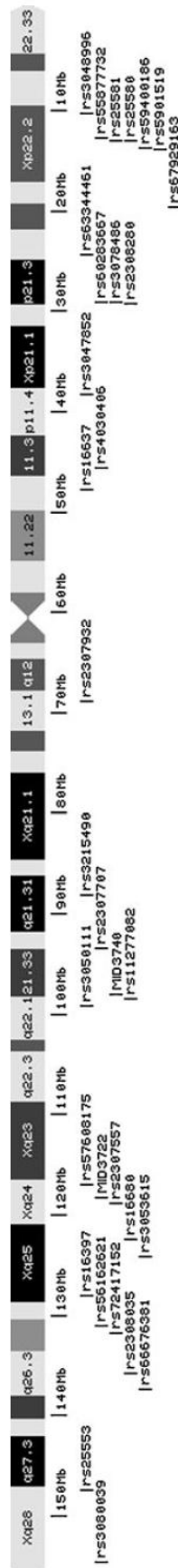
Soon after having been identified as one of the most variable types of DNA sequence in the genome, microsatellites became the marker of choice in genome mapping, forensic genetics, population genetics and other related areas [54]. Their high polymorphism or, by other words, the high level of heterozygosity, and the fact of having usually multiple alleles, makes a contrast over unique DNA [66]. Keeping in mind that males are hemizygous for all X chromosomal markers, their haplotypes are directly revealed from STR typing [67].

In the forensic field, the study of X-STR markers is emerging as an important tool in complex kinship cases not only to complement information derived from more commonly used autosomal STRs, mtDNA and Y-chromosomal variations, but also to resolve cases that otherwise would remain unsolved [45, 68-72]. The forensic interest, has prompted, in the last few year years, the development and validation of a considerable number of panels of STR markers on the X chromosome (X-STRs) usually resorting to multiplex reactions that range from six to twelve STRs per reaction [73-83].

The X-STRs panels are advantageous in deficient paternity cases, where the alleged father cannot be typed [84], in helping, for example, to determine the relationship between females presumed to share the same father.

One of the characteristics inherent to the X chromosome is that it only recombines during female meiosis. This will impact in the amount of linkage disequilibrium (LD) - that is, the non-random association between alleles at different loci, which expectedly will be higher at the X chromosome compared to autosomes. Furthermore, when dealing with X chromosome, the physical distance between loci limits the availability of independent transmitted markers. So, both linkage and LD have to be considered carefully in all X-chromosomal analyses, including those implying the interpretation and calculation of probabilities in relationship testing. To account for LD, estimates of haplotype frequencies need to be obtained and accordingly used in the analyses [45, 85, 86].

Knowing that LD serves as a theoretical foundation for association mapping, naturally a marker and a functional locus need to be in LD for the association to be detected. In the field of association studies, small isolates and admixed populations embody desired profiles for gene mapping approaches because the first ones tend to display high levels of LD due to genetic drift effects and the second ones because their gene pool is formed by relatively recent events involving distinct parental groups, which may result in long-range LD [87]. In fact, and according to the theoretical predictions, the extent of LD is influenced mostly by genetic drift, admixture and inbreeding, among others factors [88].



Similarly to other groups of genetic markers, the X-chromosome markers need population frequency databases in order to be used in forensic investigations. It is even more crucial to estimate allelic frequency distributions in different geographic areas for X-STRs, as it has already been shown that microsatellites of the sex-chromosomes are more likely to reveal population substructure than autosomal markers [89].

2.3.2. Insertion/Deletion Polymorphisms (Indels)

Whereas the patterns of genetic variation have been mainly determined by studies based on short tandem repeats (STRs) or single nucleotide polymorphisms (SNPs), more recently those makers were followed by insertion and deletion polymorphisms (Indels) [47-49].

An estimate reveals Indels are likely to represent between 16% and 25% of all sequence polymorphism in humans, making them one of the most abundant class of DNA polymorphisms, right after SNPs [90-92]. Out of these percentages, 8% are biallelic Indels [93]. Despite having received little attention up until the more recent years, these slow-evolving biallelic short insertion-deletion polymorphisms have already been recognized as a major source of evolutionary information [94].

The biallelic Indels tend to have a high range in the length difference between alleles, exhibiting, in some rare cases, the length difference of ten or even hundreds of kilobase pairs [93, 95]. Still, the largest group of Indels includes those with length differences of relatively few nucleotides [93].

Combining advantageous characteristics from both SNPs and STRs, Indels possess several features that turn them into an additional tool in population and forensic genetic studies [96]. For one, they display a widely spread distribution throughout the genome and derive from a single mutation event, occurring at a low frequency, which becomes stable. As widely noted, they can be considered as ancestry informative markers, due to displaying amid geographically separated population groups significant differences in allele frequencies. Most importantly, small Indels can be analyzed in short amplicons, allowing the improvement of the amplification of degraded DNA, and are relatively easy to genotype [96].

Despite the fact that the use of Indels is still recent, there is a clear increase of the number of studies based on Indels that have been published in the last few years, with several different purposes, one of the major being ancestry affiliation [97]. Importantly, Indels are very informative to deal with the study of genetic structure of human populations [98,99].

Evidence of this is the Weber *et al.* [93] study in 2002, which reported the identification and characterization of 2.000 biallelic Indels, by obtaining their allelic frequencies in Europeans, Africans, Japanese and Native Americans. Later, in 2006, Mills *et al.* [90] identified five major classes of Indels, and described an initial map of human Indel variation containing over 415,000 Indel polymorphisms. Of these, 35.7% were identified within known genes, and in some cases in promoters and exons of genes, where gene function is expected to be greatly influenced.

Recently, Pereira *et al.* [47] described an X-Indel multiplex system designed to amplify 32 biallelic markers in one single PCR (Figure 5). This multiplex includes X- Indels described with a high degree of polymorphism in the major human population groups: Africa, Europe and Asia. The study shows that it is possible to combine a short amplicons approach along with simplicity of analysis and good multiplexing capacity in one single reaction.

2.4. Objectives

As part of a broader project, this work sets out to study the X chromosome and characterize the populations of Miranda do Douro and Zamora through the analysis of X-STRs and X-Indels. It will be evaluated whether any correspondence exists between their genetic composition and the geographical, historical and linguistic affinities between the two regions.

So, it is important to:

- Determine the current composition of their X-chromosomal genetic pool
- Examine the micro-geographic substructure
- Assess the degree of population exchanges along the Portuguese/Spanish border, in this specific region
- Investigate the impact of geographic and political barriers in the language and in the microdifferentiation of the Leonese-speaking populations
- Contribute to further expand databases, whose data are currently insufficient.

3. Materials and Methods

3.1. DNA Samples

Two samples from unrelated individuals born in the Zamora province (Spain) and in the Miranda do Douro municipality (Portugal) were obtained in regional medical centers. For all voluntary donors, appropriate informed consent and the birth place of all their known ancestors, up to the third generation, were obtained in personal inquiries under strictly confidential circumstances. A total of 124 individuals from Miranda do Douro (69 females and 55 males) and 219 individuals from Zamora (89 females and 130 males) were considered for the present study. These samples were then verified according to their ancestry information, by only considering individuals that had origins, up to the third generation, in each region.

The Zamora province sample was divided into several regions, in order to obtain a geographical sampling resolution comparable to that available in Miranda do Douro. Six regions were considered: Aliste, Bajo-Douro, Benavente, Campos-Pan, Sanabria and Sayago. Their genetic relationships with Miranda do Douro were independently assessed.

3.2. DNA extraction

Total DNA was extracted from blood samples using the JETQUICK Blood & Cell kit (Genomed, GmbH, Germany) according to the manufacturer's specifications, in the case of the Zamora sample, and using the standard Chelex [100] or Phenol-chloroform [101] methods for the Miranda do Douro sample.

3.3. X Chromosomal markers

3.3.1. Amplification of the X-STRs

The DNA amplification for the X-chromosomal STR markers was performed using the Investigator Argus X-12 Kit (Qiagen GmbH, Hilden, Germany)[102]. This kit contains primers for 12 loci: Amelogenin (AM) for gender-determination together with DXS7132, DXS7423, DXS8378, DXS10074, DXS10079, DXS10101, DXS10103, DXS10134, DXS10135, DXS10146, DXS10148, and HPRTB (Figure 6 and Table 1).

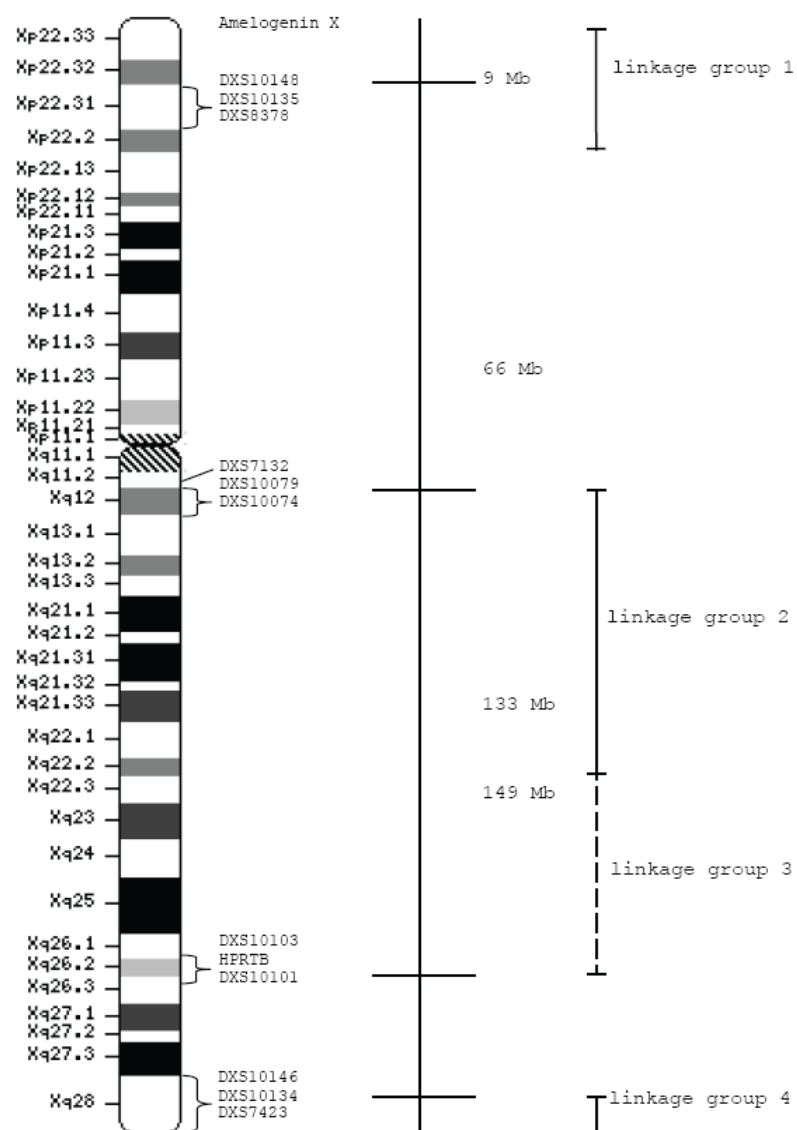


Figure 6 The ideogram of the X-chromosome describes the physical localization of the STR loci which can be analysed with the Investigator Argus X-12 Kit [Extracted from Investigator X-12 Handbook 2010, Qiagen].

Table 2 Characteristics of the 12 X-STR markers used [Partly extracted from Investigator Argus X-12 Handbook 2010].

Locus	Repeat motif of the reference allele	Reference allele
DXS7132	[TCTA] ₁₃	13
DXS7423	[TCCA] ₃ TCTGTCCT[TCCA] ₁₂	15
DXS8378	[CTAT] ₁₂	12
DXS10074	[AAGA] ₁₄	14
DXS10079	[AGAG] ₃ TGAAAGAG[AGAA] ₁₇ AGAG[AGAA] ₃	21
DXS10101	[AAAG] ₃ GAAAGAAG[GAAA] ₃ A [GAAA] ₄ AAGA [AAAG] ₅ AAAAAGAA[AAAG] ₁₃ AA	28.2
DXS10103	[TAGA] ₂ CTGA[CAGA][TAGA] ₁₁ [CAGA] ₄ [TAGA]	19
DXS10134	[GAAA] ₃ GAGA[GAAA] ₄ AA [GAAA]GAGA [GAAA] ₄ GAGA [GACAGA] ₃ [GAAA] GTAA[GAAA] ₃ AAA[GAAA] ₄ AAA[GAAA] ₁₅	35
DXS10135	[AAGA] ₃ GAAAG [GAAA] ₂₀	23
DXS10146	[TTCC] ₃ T [TTCC] ₃ TTTC CTCCCTTCC[TTCC] [TCCC]TTCTTCTTTC[TTCC] ₂ TTCTTT[CTTT] ₂ CTTC[CTTT] ₁₀ T [CTTT] ₂	26
DXS10148	[GGAA] ₄ [AAGA] ₁₂ [AAAG] ₄ N ₈ [AAGG] ₂	22
HPRTB	[AGAT] ₁₂	12

The mix of reagents (master mix) and thermocycling conditions used for this amplification are described in tables 2 and 3, respectively. The reaction was carried out in a final volume of 10 µL (9.5 µL of master mix + 0.5 µL of template DNA). A positive control sample with a known genetic profile was included in each PCR run. The positive control used was the sample *DNA XX28* (1:10) supplied with the kit (Figure 7). The runs also included a negative control where the template DNA was substituted with water, in order to screen for any contamination that could occur.

Table 2 Set up of the master mix used in the amplification with the Investigator Argus X-12 kit.

Multiplex Reaction	Volume per reaction (µL)
H2O	6,26
Investigator Argus X-12 PCR Reaction Mix	2
Investigator Argus X-12 Primer Mix	1
Multi Taq2 DNA Polymerase	0,24

Table 3 Thermocycling conditions used for the DNA amplification with the Investigator Argus X-12 kit.

		Temperature (°C)	Time
Incubation		94	4 min
5 cycles	Denaturation	96	30 sec
	Annealing	63	2 min
	Extension	72	1 min 15 sec
25 cycles	Denaturation	94	30 sec
	Annealing	60	2 min
	Extension	72	1 min 15 sec
Final extension		68	60 min

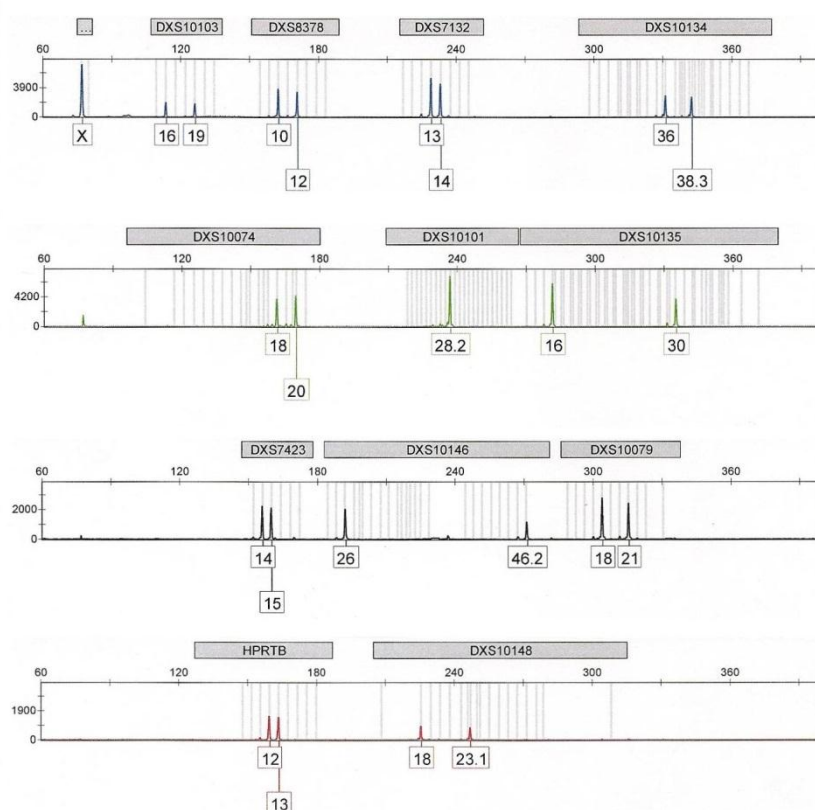


Figure 7 Electropherogram of the control sample DNA XX28 for the 12 X-STRs.

3.3.2. Amplification of the X-Indels

The amplification of 32 X-chromosomal Indel markers was performed according to Pereira *et al.* [47] (Table 4).

Table 4 Characteristics of the 32 X chromosome Indels used [Extracted from Pereira *et al.* (2011)].

	Position (bp)	Alleles	rs number
MID2612	10,234,839	-/ATC	rs3048996
MID3712	12,572,196	-/GAA	rs55877732
MID357	12,912,861	-/TGAGA	rs25581
MID356	12,918,048	-/CTT	rs25580
MID3703	13,711,300	-/GTTA	rs59400186
MID3774	13,809,000	-/ACC	rs5901519
MID3692	19,516,252	-/CATAT	rs67929163
MID3716	24,235,114	-/GAG	rs63344461
MID3690	28,984,076	-/TCAC	rs60283667
MID3719	29,040,938	-/TTAACT	rs3078486
MID2089	29,157,973	-/TTA	rs2307932
MID2692	38,262,701	-/ATT	rs3047852
MID3701	45,539,201	-/ATTA	rs4030406
MID198	47,680,386	-/CAACCAAT	rs16637
MID1736	68,733,480	-/ATA	rs2307932
MID3730	88,009,689	-/GACA	rs3215490
MID1511	93,392,006	-/GTCT	rs2307707
MID3740	97,906,546	-/GT	-
MID3732	98,331,815	-/ACCTCACTCA	rs11277082
MID3727	99,165,489	-/TT	rs3050111
MID3754	116,901,987	-/GGTCATCACGAG	rs57608175
MID3722	118156,157	-/AAAGTGTACACAT	-
MID1361	118,748,515	-/ACA	rs2307557
MID243	122,370,414	-/TGT	rs16680
MID2637	124,135,529	-/CT	rs3053615
MID111	127,958,384	-/GTG	rs16397
MID3736	130,975,547	-/CT	rs56162621
MID3753	131,760,172	-/GTATAT	rs72417152
MID1839	135,695,920	-/CA	rs2308035
MID3760	137,369,795	-/TTAAA	rs66676381
MID329	147,393,784	-/TACTCT	rs25553
MID2652	154,561,961	-/TAA	rs3080039

The reaction consisted in a single multiplex PCR using Qiagen Multiplex PCR kit (Qiagen) which is further detailed in table 5. In this case, 9 µL from the final mix were used per 1 µL of the DNA template, in a final volume of 10 µL. The thermocycling conditions used are described in table 6. A positive and a negative control were also included in all PCR runs. The genotype for the positive control – female reference sample 9947A (Promega) – is displayed in figure 8.

Table 5 Set up of the mix used in the amplification of 32 X-Indels with the Qiagen Multiplex PCR kit.

Multiplex Reaction	Volume per reaction (μL)
H2O	3
Qiagen Multiplex PCR kit	5
Qiagen Primer Mix	1

Table 6 Thermocycling conditions used for the 32 X-Indel multiplex kit.

		Temperature (°C)	Time (minutes)
Incubation		95	15 min
30 cycles	Denaturation	94	30 sec
	Annealing	60	1 min 30 sec
	Extension	72	45 sec
Final extension		72	60 min

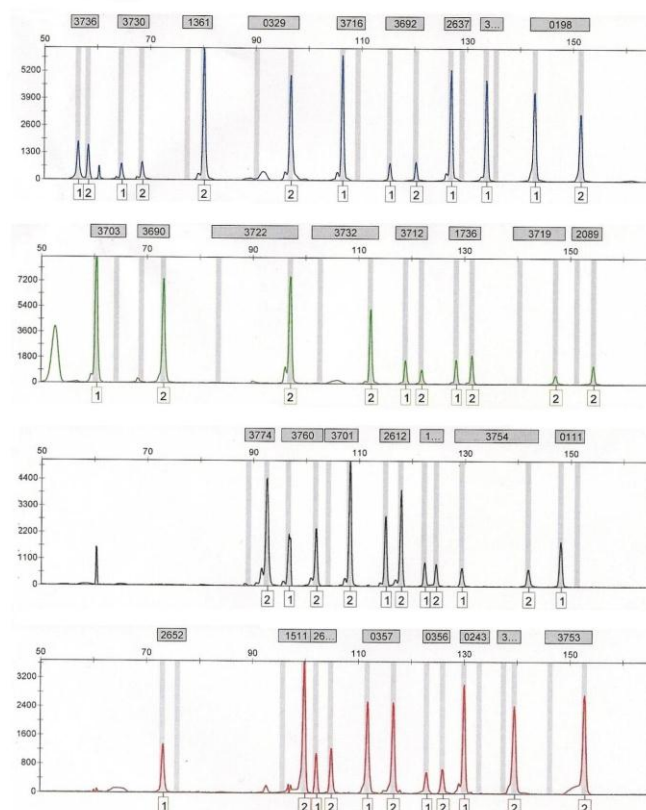


Figure 8 Electropherogram of the control sample 9947A for the 32 X-Indels.

3.3.3. Fragment detection

The PCR products obtained were prepared for subsequent analysis by adding the amplified product to Hi-Di™ Formamide (Applied Biosystems) and an internal size standard (Table 7). For the X-STR analysis a mix was prepared using 1375 µL of Hi-Di™ Formamide plus 25 µL of Size Standard 550 (BTO). An allelic ladder was also applied at this time, to help deduce the genotypes (figure 9). As for the X-Indel analysis the mix included 825 µL of Hi-Di™ Formamide and 15 µL of size standard GeneScan™ Liz 500®.

Table 7 Set up used in the capillary electrophoresis of the amplification products with the Investigator Argus X-12 Kit and 32 X-indel kit.

Samples			Ladder	
		Volume		Volume
X-STRs	Amplified Product	0.5 µL	Ladder	1 µL
	Hi- Di™ Formamide + Size Standard 550 (BTO)	10 µL	Hi- Di™ Formamide + Size Standard 550 (BTO)	10 µL
X-Indels	Amplified Product	1 µL		
	Hi-Di™ Formamide + Size Standard GeneScan™ Liz 500®.	12 µL		

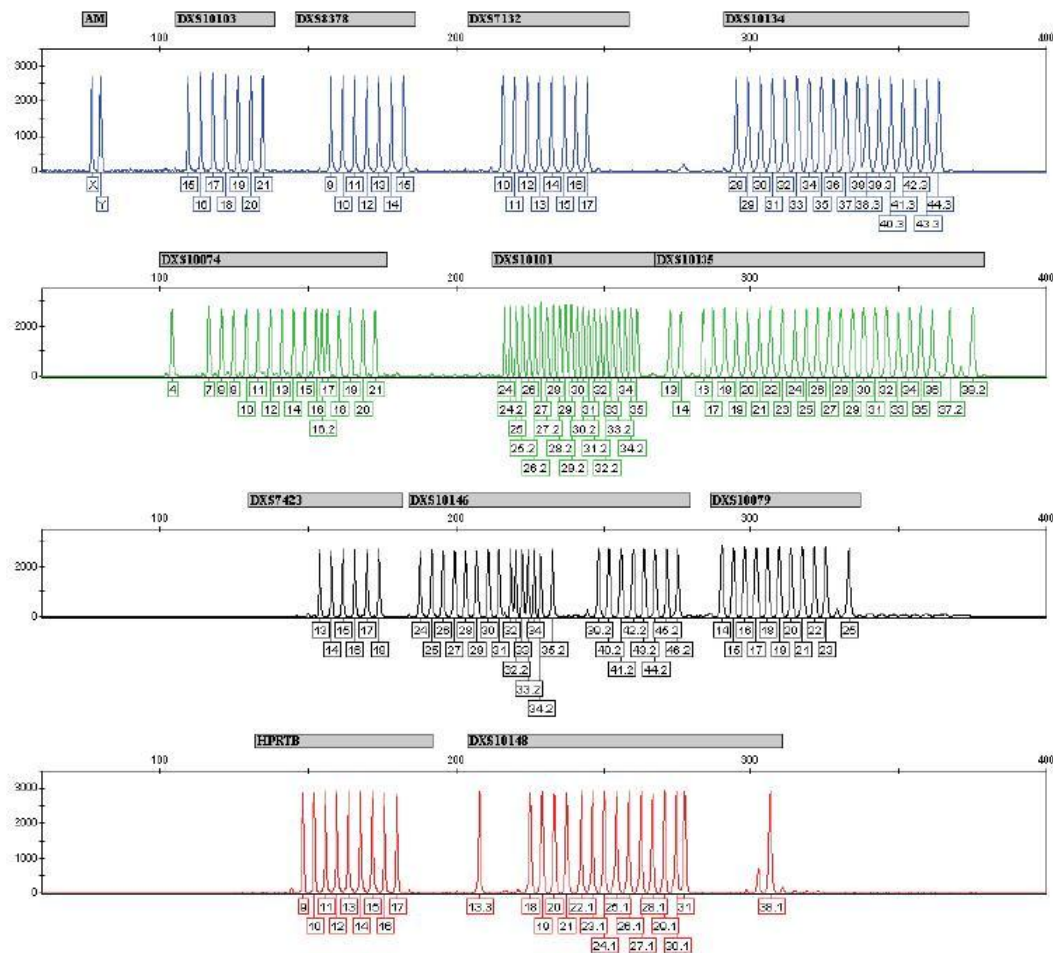


Figure 9 Electropherogram of the allelic ladder Argus X-12 analyzed on an ABI PRISM 310 Genetic Analyzer [Adapted from the Investigator Argus X-12 Kit Handbook 2010].

The PCR products were then separated by capillary electrophoresis in a ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The resulting electropherograms were analysed and genotypes were assigned with GeneMapper 4.0 software (Applied Biosystems).

3.3.4. Decaplex amplification

Two of the females studied, one from Miranda do Douro and one from Zamora presented an unclear genotypic profile in loci DXS7423 and DXS10146. In order to try to resolve the ambiguity, the samples were genotyped with a different X-STR kit (*Inhouse* developed Decaplex [40]) that comprises 10 X-STR loci (Figure 10), including the DXS7423 marker.

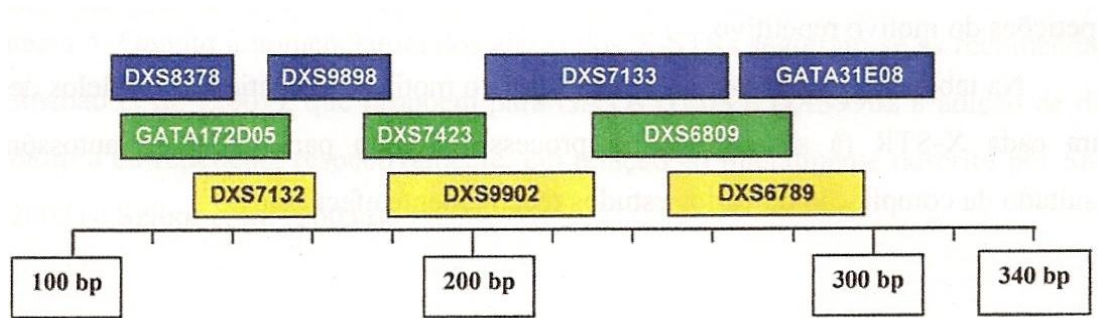


Figure 10 Multiplex used for the 10 X-STRs, with reference to the interval sizes expected for each marker in base pairs (bp). Also represented are the fluorescent colours attributed to each marker (blue: 6-FAM; green: VIC; yellow: NED)

3.4. Statistical Analysis

The information from the genotyping of STR and Indel markers was analysed with Arlequin v.3.5.1.2 software [103] in order to obtain statistical parameters as well as to assess intra and inter-population variation.

3.4.1. Intrapopulation analysis

Gene diversities [104], the mean number of pairwise differences [105] as well as allele frequencies were estimated. Female and male allele frequencies of each population were compared for significant differences in allele frequency distributions. Whenever confirmed the absence of statistical significant differences between the two sexes/genders, the individuals were pooled.

The possible deviations from Hardy-Weinberg [106] expectations were evaluated with the information provided by the female genotypes of each region.

For the linkage disequilibrium [107] analysis, only the haplotypes of males of each population were used. In this case, the number of steps in Markov chain used was of 1,000,000 and the number of dememorization steps of 10,000.

The correction for multiple tests was performed according to Bonferroni in all the analyses [108]. The Bonferroni correction is used to reduce the chances of obtaining false-positive results when multiple pair wise tests are performed on a single set of data. This method consists of dividing the critical p value by the number of comparisons being executed.

3.4.2. Comparative analysis

In order to gain a more complete view of the genetic diversity pattern of the regions of Zamora and Miranda do Douro, the results were then compared to data reported for other populations.

The pairwise F_{ST} genetic distances [109, 110] were calculated between the samples using the Arlequin v.3.5.1.2 software and POPTREE2 [111].

For X-STRs, data available are mainly from the Iberian Peninsula (Portugal and Galicia) and several European and African countries that could have contributed to the genetic compositions of these two Iberian populations (Table 8). For the comparisons between Miranda do Douro and Zamora, the information provided by the complete set of markers was considered. The remaining comparisons were based on 8 markers, since there was only available data for 8 out of the 12 X-STR markers analysed (DXS8378, DXS7423, DXS7132, HPRTB, DXS10101, DXS10134, DXS10135, DXS10074).

In the case of the X-Indels, results were compared with previously reported data for the 32 X-Indel markers from Portugal, East Africa, Somali and Iraq (Table 8). According to Pereira *et al.* (2012) [47], the population from East Africa contains male individuals from Angola and Mozambique, once assessing no statistical significant differentiation between these two populations was observed.

Table 8 Samples used in the comparative analysis of X-STRs and X-Indels.

	Country/Population		Number of individuals	Reference
X-STRs	Portugal (pt)		223	Cainé <i>et al.</i> (2012) [112]
	Portugal (po)		243	Unpublished data
	Galicia (gl)		163	Besada <i>et al.</i> (2012) [113]
	Italy (it)		160	Inturri <i>et al.</i> (2011) [114]
	Hungry (hu)		384	Zalán <i>et al.</i> (2005)/(2008) [115,116]
	Poland (pl)		311	Luczak <i>et al.</i> (2010) [117]
	Finland (fi)		300	Hedman <i>et al.</i> (2009) [118]
	Germany (ge)		1037	Edelmann <i>et al.</i> (2012) [119]
	Somali (so)		300	Hedman <i>et al.</i> (2009) [118]
	Algeria (ag)		210	Bekada <i>et al.</i> (2009) [74]
	Ghana (ga)		182	Thiele <i>et al.</i> (2008) [120]
	Morocco	Arabic (ar)	54	Bentayebi <i>et al.</i> (2012) [121]
		Berber (be)	48	
		Sahrawi (sh)	43	
	Ivory Coast (ic)		125	Pasino <i>et al.</i> (2011) [122]
	US	African (aa)	853	Diegoli <i>et al.</i> (2011) [123]
		Asian (as)		
		Caucasian (cn)		
		Hispanic (hi)		
	Chinese (ch)		272	Zeng <i>et al.</i> (2011) [124]
X-Indels	Portugal		324	Pereira <i>et al.</i> (2012) [47]
	East Africa (ang-moz)		116	
	Somali (so)		162	
	Macau (ma)		100	
	Iraq (ir)		136	Pereira <i>et al.</i> (2011) [125]

In addition, graphical representations of pairwise F_{ST} genetic distances in Multidimensional Scaling (MDS) plots were performed with the programme *STATISTICA* (StatSoft).

In order to investigate the relation between the Miranda do Douro and Zamora populations and the effects of natural or human boundaries on the genetic structure, an Analysis of MOlecular VAriance (AMOVA) [126] was conducted with the Arlequin v.3.5.1.2 software, by grouping the population samples according to three different types of barriers: political, geographical and linguistic.

Besides the samples from Miranda and Zamora, a sample from Portugal was also tested in each set, for 8 X-STRs a sample from Besada *et al.* (2012) [113] and for 32 X-Indels from Pereira *et al.* (2012) [47].

The political barrier was composed by 2 different groups: 1) regions from Zamora and 2) Miranda do Douro and Portugal. The geographical barrier divided 2 groups based on their positioning in relation to the Douro River: North of the river (Aliste, Benavente, Campos-Pan, Sanabria, Miranda do Douro and Portugal) and South of the river (Bajo-Douro and Sayago). Finally, the linguistic barrier was comprised of 3 groups according to the known distribution of the Leonese dialect: 1) Miranda do Douro, Aliste and Sanabria, 2) Portugal and 3) the remaining regions.

Another test was performed using the Barrier software v2.2, which implements the Monmonier algorithm [127] to calculate barriers relating geography and the pairwise F_{ST} genetic distance obtained in the 12 X-STR analyses. To assess the robustness of the calculated barriers, 100 resampled bootstrap data sets were generated.

4. Results and Discussion

In this study two samples of unrelated individuals from Miranda do Douro and from Zamora were characterized for 12 X-STR markers as well as for 32 X-Indel polymorphisms. Concerning X-STRs, 121 subjects from Miranda and 201 from Zamora (Aliste n=39, Bajo-Duero n=27, Benavente n=36, Campos-Pan n=48, Sanabria n=20 and Sayago n=31) were successfully typed, while for the X-Indels, data was obtained for 120 individuals from Miranda and 201 from Zamora (Aliste n=39, Bajo-Duero n=27, Benavente n=37, Campos-Pan n=47, Sanabria n=20 and Sayago n=31).

4.1. *Miranda do Douro and Zamora diversity*

4.1.1. *X-STRs*

As previously mentioned in section Material and Methods, when the 12 X-STR kit was used to perform the genotypings, two of the females analysed revealed an allele falling out the range of allelic ladders used, being uncertain whether such allele belonged to loci DXS7423 or DXS10146 (Figure 11). This result would lead to an additional study that involved the use of a different X-STR kit comprising 10 X-STRs, including DXS7423 marker but not DXS10146. Since with this alternative kit, the females revealed to be homozygous for DXS7423, the ambiguous allele initially detected with the 12 X-STR kit, could be automatically eliminated as being from the other marker, resolving therefore the conflict and helping to deduce the genotype of the samples (Figure 12).

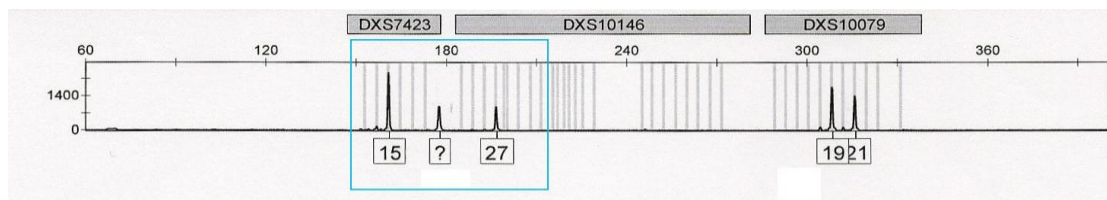


Figure 11 Detail of the electropherogram of one of the samples presenting ambiguous allele for the 12 X-STRs kit.

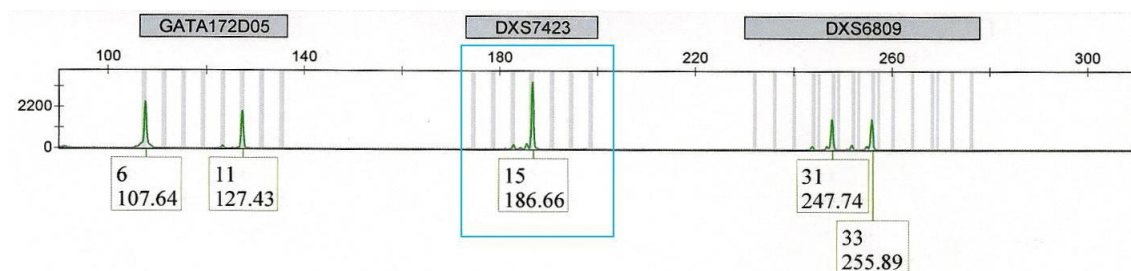


Figure 12 Detail of the electropherogram of the sample showed in figure 11 for the Decaplex kit (10 X-STRs).

Additionally, another female from Zamora presented a triallelic pattern at DXS10079 (Figure 13). After reconfirming this result through several independent PCR amplifications, the pattern was then more deeply analysed. Triallelic patterns tend to fall into one of two types: type I, where a somatic mutation of one allele occurs during an individual's development, resulting in a chimera with some cells containing the original allele and others the mutant allele; and type II, which might involve either a duplication event located in the genomic region encompassing the marker showing triallelism or a chromosomal aneuploidy. While type I patterns are characterized by uneven peak heights for the two variants of the mutated allele that sum to the height of an unmutated allele, type II patterns are associated to multiple alleles with balanced peaks that usually appear of equal height [128].

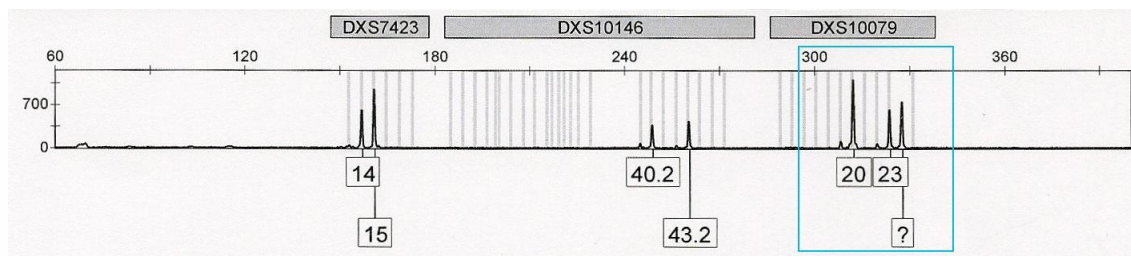


Figure 13 Detail of electropherogram of the triallelic sample (12 X-STRs).

In this case, the profile shows two smaller peaks (23 and 24) and a third with a clearly bigger peak height (20). This suggests a type I triallelic pattern, which can indicate that this individual might be carrier of a somatic mutation.

No other uncommon genotypic profiles were observed.

The allelic frequencies estimated in Miranda and Zamora for each X-STR marker are displayed in table 9. Considering the different alleles detected at each marker loci, none of them was new since all had been previously described.

As expected, all markers revealed to be highly polymorphic in both populations. Accordingly, no X-STR haplotype was shared between any of the individuals analysed.

Table 9 Allele frequencies and heterozygosity for 12 X-STRs in Miranda and Zamora.

DXS10103			DXS10134			DXS10074		
Allele	Miranda	Zamora	Allele	Miranda	Zamora	Allele	Miranda	Zamora
15	0.0267	0.0105	30		0.0070	7	0.0749	0.0245
16	0.0374	0.0699	31		0.0105	8	0.1979	0.1364
17	0.0695	0.1469	32	0.0108	0.0035	9	0.0054	0.0210
18	0.2300	0.2063	33	0.0432	0.0245	13	0.0107	0.0035
19	0.4760	0.4441	33.1		0.0035	14	0.0160	0.0140
20	0.1444	0.1014	34	0.0811	0.0804	15	0.0802	0.1014
21	0.0160	0.0210	34.2		0.0070	16	0.1711	0.2133
H _{exp}	0.6963	0.7255	35	0.2162	0.2028	17	0.2246	0.2343
DXS8378			35.3		0.0035	18	0.2032	0.1469
Allele	Miranda	Zamora	36	0.1730	0.2133	19	0.0107	0.0909
9		0.0105	36.2		0.0105	20	0.0054	0.0105
10	0.3476	0.3497	37	0.1514	0.1643	21		0.0035
11	0.3369	0.3427	37.2		0.0070	H _{exp}	0.8317	0.8425
12	0.2888	0.2797	37.3	0.0378	0.0175	DXS10101		
13	0.0267	0.0175	38	0.0757	0.0804	Allele	Miranda	Zamora
H _{exp}	0.6852	0.6841	38.2		0.0035	24.2		0.0035
DXS7132			38.3	0.0108	0.0140	25		0.0035
Allele	Miranda	Zamora	39	0.0162	0.0175	26.2	0.0054	0.0210
11	0.0160	0.0210	39.3	0.0595	0.0350	27	0.0054	0.0070
12	0.1016	0.1119	40.3	0.0865	0.0455	27.2	0.0695	0.0839
13	0.3636	0.2692	41.3	0.0162	0.0245	28	0.0695	0.0664
14	0.2995	0.3811	42.3	0.0054	0.0140	28.2	0.0856	0.0874
15	0.1711	0.1748	43.3	0.0162	0.0070	29	0.0695	0.0385
16	0.0428	0.0385	45.3		0.0035	29.2	0.0963	0.1014
17	0.0054	0.0035	H _{exp}	0.8775	0.8705	30	0.0856	0.0734
H _{exp}	0.7403	0.7398				30.2	0.1979	0.1434
DXS7423						31	0.0374	0.1084
Allele	Miranda	Zamora				31.2	0.1016	0.0979
12		0.0035				32	0.0909	0.0664
13	0.0535	0.0944				32.2	0.0428	0.0280
14	0.4278	0.2727				33	0.0267	0.0559
15	0.3369	0.4930				33.2	0.0107	0.0070
16	0.1658	0.1259				34	0.0054	0.0035
17	0.0160	0.0105				35		0.0035
H _{exp}	0.6765	0.6600				H _{exp}	0.9046	0.9162

DXS10135			DXS10146			HPRTB		
Allele	Miranda	Zamora	Allele	Miranda	Zamora	Allele	Miranda	Zamora
17	0.0321	0.0246	22	0.0054	0.0035	8	0.0054	
17.1		0.0070	24		0.0175	10		0.0070
18	0.0374	0.0316	25	0.0214	0.0490	11	0.1123	0.1399
18.1		0.0105	26	0.0963	0.0839	12	0.3476	0.3776
19	0.0535	0.0211	27	0.1230	0.1154	13	0.3797	0.2692
19.1	0.0214	0.0175	28	0.1604	0.1573	14	0.1070	0.1504
20	0.0642	0.0947	29	0.2460	0.1748	15	0.0428	0.0385
20.1	0.0160	0.0070	30	0.0588	0.0979	16	0.0054	0.0175
21	0.0588	0.0702	31	0.0267	0.0385	H _{exp}	0.7129	0.7435
21.1	0.0267	0.0211	32		0.0140	DXS10148		
22	0.1016	0.1053	34.2	0.0054		Allele	Miranda	Zamora
22.1	0.0054	0.0386	35.2		0.0035	13.3		0.0035
23	0.0963	0.0737	36.2		0.0105	17	0.0215	0.0035
23.1	0.0214	0.0211	38.2	0.0107	0.0140	18	0.1344	0.1259
24	0.0802	0.1088	39.2	0.0321	0.0315	19	0.0215	0.0315
25	0.0428	0.0632	40.2	0.0428	0.0630	20	0.0054	0.0070
25.1		0.0035	41.2	0.0374	0.0420	20.1	0.0323	
26	0.0588	0.0526	42.2	0.0321	0.0175	21		0.0035
27	0.0321	0.0597	43.2	0.0321	0.0175	22		0.0140
28	0.0749	0.0632	44.2	0.0535	0.0245	22.1	0.0376	0.0315
29	0.0428	0.0316	45.2	0.0107	0.0140	23	0.0430	0.0664
30	0.0321	0.0281	46.2		0.0070	23.1	0.0430	0.0490
31	0.0267	0.0246	47.2	0.0054	0.0035	24	0.0215	0.0210
32	0.0054	0.0105	H _{exp}	0.8799	0.9050	24.1	0.0645	0.1608
32.1		0.0035	DXS10079			25	0.0108	
33	0.0588	0.0035	Allele	Miranda	Zamora	25.1	0.1344	0.1399
34	0.0107		15	0.0481	0.0175	26.1	0.1183	0.1818
H _{exp}	0.9451	0.9393	16	0.0428	0.0385	27.1	0.1344	0.1119
			17	0.0909	0.0559	28	0.0054	
			18	0.1497	0.1643	28.1	0.1290	0.0280
			18.2		0.0035	29.1	0.0215	0.0105
			19	0.2353	0.2587	30.1	0.0215	0.0070
			20	0.2941	0.2587	32.1		0.0035
			21	0.0856	0.1434	H _{exp}	0.9072	0.8858
			22	0.0481	0.0525			
			23	0.0054	0.0070			
			H _{exp}	0.8180	0.8137			

The highest variability was observed for the system DXS10135, for which a total of 27 alleles were observed (most of them shared by the two populations) accounting for a heterozygosity of 0.9451 and 0.9393 in Miranda do Douro and Zamora, respectively.

On the opposite range of diversity, was the X-STR DXS7423, showing quite few alleles and a heterozygosity of 0.6765 in Miranda and 0.65999 in Zamora.

Overall, in terms of loci diversities, the results here obtained fit well previous descriptions for the same set of markers [45].

4.1.1.1. Hardy-Weinberg Equilibrium

For each marker, the genotypic distribution observed in females was tested for Hardy-Weinberg Equilibrium (HWE) and the obtained results are shown in table 10. Only three p values below 0.05 were found: for DXS10134 and DXS10148 in Miranda ($p=0.02477$; $p=0.01782$), and for DXS10146 in Zamora ($p=0.02313$). However, since several tests were performed, the Bonferroni correction for multiple tests needed to be applied, giving in this case a significance value of 0.0042. Under this level, none of the obtained p values were significant, meaning that no deviations from the HWE were detected in the distributions of female genotypes in each population for these markers.

Table 10 Results of HWE test for 12 X-STRs, in a sample of 66 females from Miranda and 86 females from Zamora.

Marker	Miranda do Douro			Zamora		
	Obs.Het.	Exp.Het.	P -value \pm s.d.	Obs.Het.	Exp.Het.	P -value \pm s.d.
DXS10103	0.65152	0.69651	0.58224 ± 0.00052	0.67059	0.73637	0.28043 ± 0.00044
DXS8378	0.68182	0.68945	0.26922 ± 0.00042	0.70588	0.68131	0.84931 ± 0.00040
DXS7132	0.78788	0.74774	0.95157 ± 0.00022	0.76471	0.74849	0.68724 ± 0.00047
DXS10134	0.80000	0.88145	0.02477 ± 0.00011	0.91765	0.87233	0.76163 ± 0.00019
DXS10074	0.81818	0.82061	0.65567 ± 0.00036	0.85882	0.85437	0.93883 ± 0.00016
DXS10101	0.90909	0.90238	0.12027 ± 0.00021	0.87059	0.91549	0.45796 ± 0.00072
DXS10135	0.90909	0.94344	0.20476 ± 0.00016	0.94048	0.93919	0.80857 ± 0.00019
DXS7423	0.68182	0.68390	0.93035 ± 0.00028	0.61176	0.66885	0.75542 ± 0.00040
DXS10146	0.90909	0.88746	0.42261 ± 0.00019	0.84706	0.91055	0.02313 ± 0.00009
DXS10079	0.83333	0.80696	0.81194 ± 0.00038	0.78824	0.80362	0.71566 ± 0.00037
HPRTB	0.74242	0.71282	0.93769 ± 0.00027	0.67059	0.74897	0.31715 ± 0.00040
DXS10148	0.81818	0.90354	0.01782 ± 0.00012	0.85882	0.88340	0.36308 ± 0.00032

Obs.Het. – observed heterozygosity; Exp.Het. – expected heterozygosity.

4.1.1.2. Genetic distance - F_{ST}

The genetic distance between the studied populations was measured through F_{STs} . First we compared Miranda do Douro and Zamora, between which the F_{ST} value was 0.005 having an associated p value of 0, meaning that this distance is statistically significant. With this, Zamora was divided into its six regions and pairwise comparisons

with Miranda were performed. In table 11 are represented the F_{ST} values obtained between these seven population' samples.

Table 11 Genetic distances (F_{ST}) between six regions of Zamora and Miranda for 12 X-STRs.

	Aliste	Bajo-Duero	Benavente	Campos-Pan	Sanabria	Sayago	Miranda
Aliste	0.0000						
Bajo-Duero	-0.0026	0.0000					
Benavente	0.0035	0.0041	0.0000				
Campos-Pan	-0.0012	0.0018	0.0072*	0.0000			
Sanabria	0.0054	0.0000	-0.0009	0.0049	0.0000		
Sayago	-0.0009	-0.0019	0.0127**	-0.0010	0.0050	0.0000	
Miranda	0.0019	0.0043	0.0101**	0.0034*	0.0120**	0.0087*	0.0000

* p value significant for 0.05; ** p value significant for 0.0071

After the Bonferroni correction for seven tests the level of significance assumed was $p \leq 0.05/7 = 0.0071$. The highest F_{ST} value observed was between Benavente and Sayago ($F_{ST} = 0.0127$), for which the pairwise p value was 0.00426, which is statistically significant. Other than this, two more p values were below the level of significance: one associated to the genetic distance between Benavente and Miranda do Douro ($p = 0.00010$) and the other to the distance between Sanabria and Miranda do Douro ($p = 0.00515$). So, since these three p values are significant, the finding suggests that some genetic differentiation, although slim, exist between the populations examined, and more importantly two of the signs of differentiation involved Miranda do Douro, which differed from two regions from Zamora. This result appears to support the relevance of the political border in the generation of genetic differentiations between the populations of the two different countries.

Comparisons were next extended to two other samples, from Portugal and Galicia, studied by Besada *et al.* (2012) [113], but lowering the level of resolution to only 8 X-STRs, which were the STRs simultaneously screened in our and in the study of Besada *et al.* Table 12 shows the F_{ST} values obtained for the nine populations.

Table 12 Genetic distances (F_{ST}) between the Zamora regions, Miranda, Portugal and Galicia for 8 X-STRs.

	Aliste	Bajo-Duero	Benavente	Campos-Pan	Sanabria	Sayago	Miranda	Portugal	Galicia
Aliste	0								
Bajo-Duero	0.0019	0							
Benavente	0.0037	0.0043	0						
Campos-Pan	-0.0007	0.0028	0.0059	0					
Sanabria	0.0055	0.0007	-0.0037	0.0069	0				
Sayago	0.0005	0.0009	0.0135*	0.0000	0.0068	0			
Miranda	0.0016	0.0070*	0.0063*	0.0042*	0.0135*	0.0147**	0		
Portugal	0.0020	0.0028	0.0004	0.0029	0.0006	0.0132*	0.0006	0	
Galicia	-0.0006	0.0027	0.0021	0.0006	0.0037	0.0053	0.0031*	0.0001	0

* p value significant for 0.05; ** p value significant for 0.0056

For $p \leq 0.05$, the values statistically significant are demonstrative of a differentiation between the Portuguese and the Spanish populations. Miranda shows a significant distance to Galicia and all the Zamora regions except Aliste. This Miranda-Aliste connection would be expected considering that in both populations the Leonese dialect is (or was) spoken, a factor that could have strengthen affinities. However, it was also expected to see a similar relation with Sanabria, the other Leonese-speaking region, which was not seen.

However, when the Bonferroni correction was adjusted for multiple tests ($p=0.0056$), only the highest F_{ST} value registered, which was between Sayago and Miranda do Douro, was associated to a significant p value ($p=0.00168$), but even so the result indicates that some differentiation exists between two adjacent geographical regions only separated by their countries border.

4.1.1.3. Linkage Disequilibrium

Since this study comprehended the analysis of markers located in the same chromosome, especial attention needed to be paid to the level of linkage disequilibrium (LD) between markers. It is important to assess whether or not there is LD in the transmission of alleles from different loci, to facilitate the proper construction of genetic databases.

In this case, we were dealing with 12 STRs, and so LD tests were performed for 66 pairs of loci. The confidence level assumed was 0.000758, which was obtained after applying the Bonferroni correction for multiple tests ($p \leq 0.05/66$). In the sample from Miranda do Douro, a significant association was detected for the pair of markers DXS10135-DXS10148 ($p=0.000373$), which are markers that belong to the same linkage group (I) in X chromosome (Figure 6). In Zamora, also two pairs of loci revealed significant levels of association: DXS10103-DXS10101 ($p=0$) and DXS10101-HPRTB ($p=0.000031$). The 3 markers involved in these two associations pertain to the linkage group III of X chromosome (Figure 6). Given that LD is more difficultly broken between loci that are linked, significant LD, although of weak magnitude, has been reported quite commonly for linked X-STRs in other populations [114,122]. So, the patterns of LD observed in Miranda or Zamora do not indicate any distinctiveness comparatively to the usually observed in most populations.

Even though, the detection of significant LD between a few markers, implies that those markers cannot be considered as independently transmitted, but instead as a small haploblock. Therefore, based on data from the males of the population samples, we have calculated, by direct counting, haplotypic frequencies for the markers' pairs in LD, and next we have recalculated the F_{ST} distances between populations, now accounting for LD (Table 13). In this analysis, only the male samples were considered.

Table 13 Genetic distances (F_{ST}) considering LD between the Zamora regions and Miranda for the 12 X-STR markers.

	Miranda	Aliste	Bajo-Duro	Benavente	Campos-Pan	Sanabria	Sayago
Miranda	0.0000						
Aliste	-0.0049	0.0000					
Bajo-Duro	0.0020	-0.0026	0.0000				
Benavente	-0.0007	-0.0043	-0.0002	0.0000			
Campos-Pan	-0.0090	0.0014	-0.0048	0.0014	0.0000		
Sanabria	-0.0044	-0.0077	-0.0101	-0.0080	0.0049	0.0000	
Sayago	0.0173*	0.0028	0.0109	0.0292**	0.0132	0.0165	0.0000

* p value significant for 0.05; ** p value significant for 0.0071

In general, values of genetic distances diminished comparatively to those obtained assuming independence between markers (Table 11).

Among the new F_{ST} distances, only one, corresponding to the genetic distance between Benavente and Sayago, remained significant after applying the multiple test correction ($F_{ST}=0.0292$; $p=0.00653$). So, the previously detected differentiation between Miranda and Benavente or Sayago was not observed in the male fraction of the populations when data was treated accounting for LD.

4.1.2. X-Indels

The allelic frequencies obtained for each of the X-Indel marker in Miranda and Zamora are displayed in table 14 as well as are the corresponding heterozygoties. Similarly to the observed for the X-STRs, no haplotype was shared between individuals within or between the populations. Most of the Indels showed diversities in the higher range of the possible values for biallelic markers (0-0.5), and only MID2637 and MID3727 presented diversities below 0.2 in one of the studied populations. Level of diversity in each marker was, however, similar in both populations.

Table 14 Allelic frequencies and heterozygosity for 32 X-Indels. Only the frequencies of the short alleles are presented.

	Mir.	Zam.		Mir.	Zam.		Mir.	Zam.		Mir.	Zam.
MID 3736	0.6703	0.6864	MID 198	0.6344	0.6307	MID 2089	0.3172	0.3263	MID 2652	0.7880	0.7631
H_{exp}	0.4444	0.4320		0.4664	0.4675		0.4355	0.4412		0.3359	0.3629
MID 3730	0.3187	0.2857	MID 3703	0.3387	0.3484	MID 3774	0.1828	0.2091	MID 1511	0.7043	0.7038
H_{exp}	0.4367	0.4096		0.4504	0.4556		0.3004	0.3319		0.4188	0.4184
MID 1361	0.2043	0.2056	MID 3690	0.3817	0.3868	MID 3760	0.7473	0.7518	MID 2692	0.7243	0.6912
H_{exp}	0.3269	0.3278		0.4746	0.4760		0.3797	0.3746		0.4015	0.4284
MID 329	0.3226	0.2544	MID 3722	0.3226	0.3554	MID 3701	0.3226	0.4007	MID 357	0.3118	0.3415
H_{exp}	0.4394	0.3806		0.4394	0.4598		0.4394	0.4820		0.432	0.4513
MID 3716	0.6989	0.7143	MID 3732	0.3172	0.2125	MID 2612	0.4355	0.4460	MID 356	0.3989	0.4000
H_{exp}	0.4231	0.4096		0.4355	0.3359		0.4943	0.4959		0.4823	0.4817
MID 3692	0.2043	0.1568	MID 3712	0.2065	0.2021	MID 1839	0.8470	0.8182	MID 243	0.8387	0.7666
H_{exp}	0.3269	0.2653		0.3295	0.3236		0.2606	0.2986		0.2720	0.3592
MID 2637	0.8710	0.8955	MID 1736	0.4785	0.4390	MID 3754	0.1398	0.2160	MID 3727	0.0968	0.1359
H_{exp}	0.2260	0.1879		0.5000	0.4943		0.2418	0.3399		0.1758	0.2357
MID 3740	0.5000	0.4112	MID 3719	0.1250	0.1544	MID 111	0.5355	0.5331	MID 3753	0.0753	0.1045
H_{exp}	0.5000	0.4859		0.2200	0.2620		0.5000	0.4996		0.1400	0.1879

Mir. - Miranda do Douro; Zam. - Zamora.

4.1.2.1. Hardy-Weinberg Equilibrium

The genotypic distributions observed in the fraction of females in each sample were tested against the Hardy-Weinberg expectations, with the results being displayed in table 15. Whilst three p values below 0.05 were found for the markers MID198, MID3722 and MID3760 in Miranda ($p=0.01411$; $p=0.00970$; $p=0.03716$), all lost the significance after applying the Bonferroni correction (the significance value was 0.0016). So, no significant deviations from the HWE were detected in the distributions of female genotypes in each population for these markers.

Table 15 Results of HWE test to the 32 X-Indels, in a sample of 66 females from Miranda and 86 females from Zamora.

Marker	Miranda do Douro			Zamora		
	Obs.Het.	Exp.Het.	$P\text{-value} \pm \text{s.d.}$	Obs.Het.	Exp.Het.	$P\text{-value} \pm \text{s.d.}$
MID3736	0.47692	0.46524	1 ± 0.00000	0.39535	0.41480	0.79344 ± 0.00040
MID3730	0.53846	0.44615	0.15780 ± 0.00037	0.39535	0.39412	1 ± 0.00000
MID1361	0.31818	0.34548	0.49069 ± 0.00052	0.31395	0.32606	0.74167 ± 0.00042
MID329	0.42424	0.42563	1 ± 0.00000	0.36047	0.41962	0.20209 ± 0.00041
MID3716	0.42424	0.41314	1 ± 0.00000	0.36047	0.36523	1 ± 0.00000
MID3692	0.30303	0.31876	0.69985 ± 0.00044	0.25581	0.24153	1 ± 0.00000
MID2637	0.28788	0.24832	0.33604 ± 0.00048	0.23256	0.20672	0.59335 ± 0.00051
MID3740	0.43939	0.50278	0.33134 ± 0.00044	0.44186	0.48552	0.50223 ± 0.00052
MID198	0.59091	0.45281	0.01411 ± 0.00012	0.45349	0.47294	0.81785 ± 0.00038
MID3703	0.45455	0.47421	0.79529 ± 0.00042	0.39535	0.45696	0.23883 ± 0.00041
MID3690	0.42424	0.47421	0.43828 ± 0.00049	0.44186	0.48552	0.50175 ± 0.00053
MID3722	0.28788	0.44263	0.00970 ± 0.00010	0.44186	0.47572	0.64694 ± 0.00046
MID3732	0.42424	0.45755	0.59272 ± 0.00049	0.30233	0.33293	0.50992 ± 0.00051
MID3712	0.24615	0.32248	0.10939 ± 0.00032	0.34884	0.34625	1 ± 0.00000
MID1736	0.56061	0.49815	0.33055 ± 0.00049	0.46512	0.48960	0.66181 ± 0.00047
MID3719	0.30769	0.26237	0.33479 ± 0.00048	0.24706	0.23536	1 ± 0.00000
MID2089	0.42424	0.45755	0.59178 ± 0.00049	0.41176	0.43167	0.80083 ± 0.00041
MID2652	0.31818	0.30939	1 ± 0.00000	0.33721	0.35271	0.75844 ± 0.00041
MID1511	0.39394	0.37011	0.74231 ± 0.00044	0.43023	0.36523	0.13587 ± 0.00033
MID2692	0.43939	0.45281	1 ± 0.00000	0.52326	0.48327	0.50396 ± 0.00052
MID357	0.40909	0.50093	0.14624 ± 0.00038	0.48837	0.50265	0.83025 ± 0.00038
MID356	0.26154	0.25152	1 ± 0.00000	0.30233	0.34625	0.34200 ± 0.00046
MID243	0.21212	0.25908	0.15015 ± 0.00034	0.33721	0.35271	0.75797 ± 0.00043
MID3727	0.41538	0.49958	0.21412 ± 0.00041	0.51163	0.50292	1 ± 0.00000
MID3753	0.33333	0.29979	0.67597 ± 0.00050	0.36047	0.33966	0.75116 ± 0.00044
MID3774	0.39394	0.43720	0.56900 ± 0.00048	0.41860	0.41480	1 ± 0.00000
MID3760	0.30303	0.41314	0.03716 ± 0.00018	0.45882	0.43167	0.62050 ± 0.00048
MID3701	0.40909	0.43153	0.77369 ± 0.00041	0.53488	0.46376	0.16884 ± 0.00040
MID2612	0.40625	0.49163	0.20006 ± 0.00039	0.58824	0.49119	0.07871 ± 0.00028
MID1839	0.22727	0.24832	0.60692 ± 0.00050	0.34884	0.39412	0.28451 ± 0.00045
MID3754	0.18182	0.16655	1 ± 0.00000	0.22093	0.23303	0.63919 ± 0.00047
MID111	0.15152	0.16655	0.42234 ± 0.00048	0.19767	0.17918	1 ± 0.00000

Obs.Het. – observed heterozygosity; Exp.Het. – expected heterozygosity.

4.1.2.2. Genetic distance - F_{ST}

When the genetic distance between the populations of Zamora and Miranda do Douro was measured, the F_{ST} value obtained was of 0.00012 with a p value of 0.39659.

Then the six regions from Zamora were compared to Miranda do Douro. In table 16 are represented the F_{ST} values obtained for pairs of these seven populations.

Table 16 Genetic distances (F_{ST}) between six regions of Zamora and Miranda for 32 X-Indels.

	Aliste	Bajo-Duero	Benavente	Campos-Pan	Sanabria	Sayago	Miranda
Aliste	0.0000						
Bajo-Duero	0.0029	0.0000					
Benavente	0.0034	-0.0007	0.0000				
Campos-Pan	-0.0079	-0.0024	0.0022	0.0000			
Sanabria	0.0036	0.0021	0.0107	-0.0021	0.0000		
Sayago	-0.0001	-0.0042	0.0063	-0.0070	0.0033	0.0000	
Miranda	0.0014	0.0009	0.0000	0.0001	0.0023	-0.0020	0.0000

All the F_{ST} values were very low, with the highest one having been observed between Benavente and Sanabria ($F_{ST}=0.0107$), but its associated p value was 0.09177, which is not significant, even without applying the correction for multiple tests ($p \leq 0.05/7=0.0071$). None of the other F_{ST} values reached statistical significance.

So, for Indels no minor signs of heterogeneity in the populations from Zamora and Miranda were detected.

As done previously for the X-STRs, we thought to extend comparisons to other populations characterized for the same set of 32 X-Indels (Table 17). Up to now, only Pereira *et al.* (2012) [47] reported data for this multiplex system, having studied different populations across the world. All the populations analysed by Pereira *et al.* were used in the comparisons. Not surprisingly, the highest F_{ST} values come from the comparison of the African populations against the Iberian ones. For example, between “Angola-Mozambique” and Sanabria the value was 0.1164 and between Somali and Miranda 0.0667. The only significant p values (significance level assuming multiple tests: $p \leq 0.05/12=0.0042$) were found in pairs involving an African and a non-African population. Also among African populations, a significant p value was observed between “Angola-Mozambique” and Somalia ($F_{ST}=0.0301$). As for the Iberian

populations, genetic distances were comparatively very low, and accordingly also statistically non-significant.

Table 17 Genetic distances (F_{ST}) between the regions of Zamora, Miranda, Portugal, Somalia, Angola-Mozambique (Ang-Moz), Macau and Iraq for 32 X-Indels.

	Aliste	Bajo-Duero	Benavente	Campos-Pan	Sanabria	Sayago	Miranda	Somalia	Ang-Moz	Portugal	Macau	Iraq
Aliste	0.0000											
Bajo-Duero	0.0029	0.0000										
Benavente	0.0034	-0.0007	0.0000									
Campos-Pan	-0.0079	-0.0024	0.0022	0.0000								
Sanabria	0.0036	0.0021	0.0107	-0.0021	0.0000							
Sayago	-0.0001	-0.0042	0.0063	-0.0070	0.0033	0.0000						
Miranda	0.0014	0.0009	0.0000	0.0001	0.0023	-0.0020	0.0000					
Somalia	0.0481**	0.0477**	0.0462**	0.0578**	0.0598**	0.0619**	0.0667**	0.0000				
Ang-Moz	0.0994**	0.0941**	0.0901**	0.1074**	0.1164**	0.1104**	0.1142**	0.0301**	0.0000			
Portugal	0.0011	0.0078	0.0057	0.0001	0.0090	-0.0003	0.0049	0.0528**	0.1036**	0.0000		
Macau	0.1084**	0.1268**	0.1444**	0.1163**	0.1267**	0.1330**	0.1373**	0.1322**	0.1594**	0.1312**	0.0000	
Iraq	0.0054	0.0088*	0.0145**	0.0084*	0.0111	0.0070	0.0141**	0.0525**	0.1026**	0.0132**	0.0791**	0.0000

* p value significant for 0.05; ** p value significant for 0.0042

4.1.2.3. Linkage Disequilibrium

LD was also assessed for the 32 X-Indels under study, which implied to perform 496 tests association tests, leading to assume a significance level of $p \leq 0.05/496 = 0.0001$. In both Miranda do Douro and Zamora, the tests for the pair MID357-MID356 presented a $p=0$, which indicate a significant association between these markers. The positions of these two Indels are 12,912,862 and 12,918,049 bps, respectively, at Xq22, which means that they are in close proximity (Physical location according to UCSC Genome Browser on Human). Therefore, the tight linkage might explain the detected association between them. For the same pair of Indels, significant LD has been sporadically observed in other populations [47,129].

As observed for the X-STRs, also for the X-Indels, neither Miranda nor Zamora presented a pattern of LD drawing any especial attention.

However, the detection of non-independent transmission between alleles of MID357 and MID356, once again implied the recalculation of the F_{ST} populations' distances, accounting for the existence of LD between the two markers (Table 18).

Table 18 Genetic distances (F_{ST}) considering LD between the Zamora regions and Miranda for the 32 X-Indel markers.

	Aliste	Bajo-Duero	Benavente	Campos-Pan	Sanabria	Sayago	Miranda
Aliste	0.0000						
Bajo-Duero	-0.0268	0.0000					
Benavente	0.0075	0.0022	0.0000				
Campos-Pan	-0.0133	-0.0078	0.0149	0.0000			
Sanabria	0.0204	-0.0380	0.0227	0.0309*	0.0000		
Sayago	-0.0016	-0.0203	0.0092	0.0139	0.0484*	0.0000	
Miranda	-0.0019	-0.0103	0.0004	0.0079	0.0161	-0.0094	0.0000

* p value significant for 0.05

Comparatively to the results obtained without correction for LD (table 16), no substantial differences were observed, although now the highest F_{ST} value was between Sanabria and Sayago. Still, no significant p values were registered.

4.1.3. STRs + INDELS

Once there was information available for two sets of different markers, X-STRs and X-Indels, both were combined, to increase the coverage of X-chromosomal markers and to expectedly achieve a high level of resolution in the study.

Within both Miranda do Douro and Zamora samples the patterns of LD on the X chromosome prompted the recalculation of gene diversities accounting for LD in each set of markers, as previously discussed in the F_{ST} calculations. The new values are also presented in Table 19.

Comparing the diversity parameters between the two populations, Miranda do Douro tends to present slightly lower diversities in all sets of markers, which can be a sign of isolation of the population over the years. More recently, the desertification of the rural areas has contributed to an increasingly losing of inhabitants in Miranda do Douro.

Therefore, the small population size of Miranda do Douro - a factor that favours genetic drift, could lead to the expectation that it should be characterised by a clear reduced

diversity. Yet, the difference between Miranda do Douro and Zamora concerning levels of diversity does not seem to be too relevant.

Table 19 Average gene diversity (H) and mean number of pairwise differences (M and the corresponding standard deviations, $M \pm s.d.$) in Miranda do Douro and Zamora populations. Corrected values for each set of markers are also presented after detection of linkage disequilibrium in several pairs.

	STRs		Indels		STRs + Indels	
	Miranda do Douro	Zamora	Miranda do Douro	Zamora	Miranda do Douro	Zamora
H	0.8039	0.8099	0.3759	0.3855	0.4874	0.4988
H_{LD}	0.8338	0.8305	0.3665	0.3823	0.4728	0.4833
M	9.6469 \pm 4.4412	9.7192 \pm 4.4654	12.0272 \pm 5.4607	12.3351 \pm 5.5849	21.4437 \pm 9.6318	21.9455 \pm 9.7367
M_{LD}	7.5039 \pm 3.5584	7.4744 \pm 3.5172	11.3605 \pm 5.2441	11.8515 \pm 5.3996	18.9103 \pm 8.5423	19.3331 \pm 8.6203

For the LD analysis of the complete set of X-chromosomal markers (in total 44 markers), 946 association tests were carried out. Under the correction for multiple tests ($p \leq 0.05/946 = 0.000053$), in Miranda do Douro, only the pair of markers, MID357-MID356, was found to be in LD ($p=0$), as had been already identified. In Zamora, besides the previously known pairs in LD DXS10103-DXS10101, DXS10101-HPRTB and MID357-MID356, a new marker association was detected between the pair HPRTB-MID2637 ($p=0.00005$). The two latter markers are also in close proximity, which again contributes to explain the detected association between the two markers. To better show these connections, figure 14 represents a map of the X-chromosomal markers in LD and their genetic location (in cM) according to Rutgers map interpolator [130]. The lowest genetic distance observed is 0.014 cM between the pair DXS10101-HPRTB, while the greatest is 8.3 cM between HPRTB-MID2637.

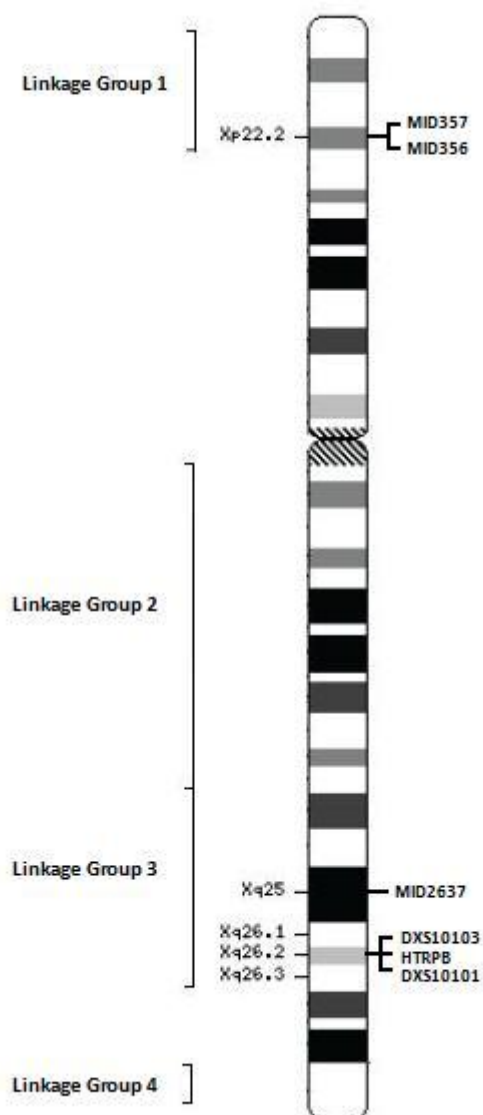


Figure 14 Map of X-chromosomal markers in close LD based on their genetic positions according to Rutgers map.

4.2. Analysis of barriers

To deepen the knowledge on the genetic history between the Miranda do Douro and Zamora populations it appeared important to try to infer the effects of natural or human boundaries on their genetic structure. In Table 20 are presented the results yielded by AMOVA when populations were grouped according to different criteria: political, geographical and linguistic.

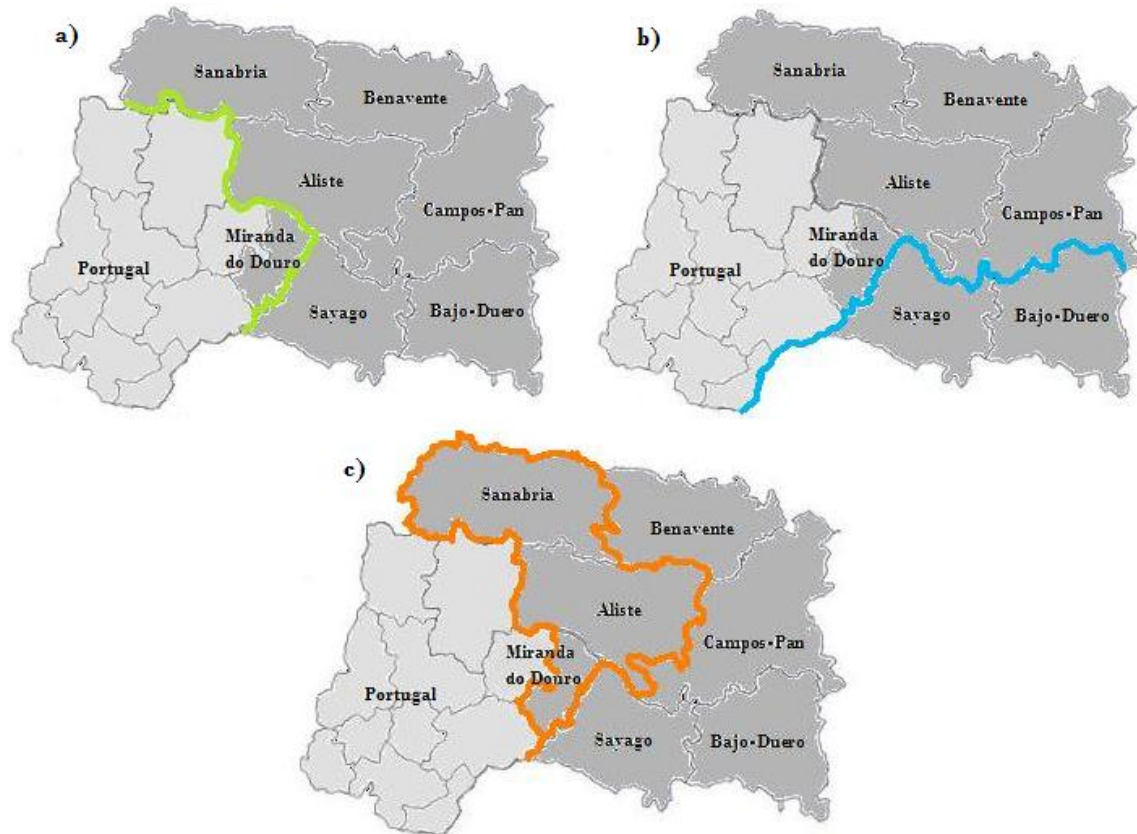


Figure 15 Maps displaying the considered barriers: a) political, b) geographical and c) linguistic.

The political division considered two main groups: Portuguese populations and Spanish populations (Figure 15a). Whilst, the geographical barrier was based on the positioning of our target regions in relation to the Douro River, considering samples as North of the river and South of the river, as the presence of a natural barrier could have had some impact in the current genetic profiles of the populations (Figure 15b). As for the linguistic barrier, the regions were grouped according to the presently known distribution of the Leonese dialects and the remaining surrounding languages, Portuguese and Castilian (Figure 15c).

Table 20 Results obtained from the AMOVA analysis, regarding 8 X-STRs and 32 X-Indels.

		Political Barrier	Geographical Barrier	Linguistic Barrier
Percentage of variation				
X-STRs	Among groups	0.36	0.35	-0.09
	Among populations within groups	0.23	0.04	0.51
	Within populations	99.42	99.25	99.58
X-INDELs	Among groups	-0.01	-0.14	0.21
	Among populations within groups	0.22	0.2	0.04
	Within populations	99.79	99.94	99.76
Fixation indices				
X-STRs	FCT	0.00355	0.00349	-0.00086
	FSC	0.00227	0.00404	0.00508
	FST	0.00581	0.00751	0.00423
X-INDELs	FCT	-0.00011	-0.00139	0.00206
	FSC	0.00221	0.00196	0.00039
	FST	0.00210	0.00058	0.00244
P-value				
X-STRs	Va and FCT	0.17910 ± 0.00271	0.07412 ± 0.00173	0.68475 ± 0.00347
	Vb and FSC	0.08351 ± 0.00201	0.00549 ± 0.00048	0.00799 ± 0.00059
	Vc and FST	0.00105 ± 0.00022	0.00070 ± 0.00018	0.00115 ± 0.00026
X-INDELs	Va and FCT	0.09954 ± 0.00195	0.85516 ± 0.00252	0.15588 ± 0.00277
	Vb and FSC	0.09954 ± 0.00195	0.10588 ± 0.00208	0.40431 ± 0.00351
	Vc and FST	0.07557 ± 0.00168	0.14529 ± 0.00274	0.07302 ± 0.00175

For X-STRs, the political and the geographical barriers account more to differences among groups of populations (0.36 and 0.35, respectively), than their linguistic affiliation, which in fact does not contribute to explain any differences among those groups. On the other hand, for X-Indels the linguistic barrier has clearly more impact in the proportion of variation due to differences among groups, than the political or geographical boundaries.

The STRs are known to have a high mutation rate, tending to better capture signs of a more recent genetic history, whilst the Indels are more stable, and consequently may trace more ancient aspects of history. This could have had some impact in the conclusions, had there been any significant *p* value for the observed differences among groups, which in reality were too small and very far from reaching statistical significance. Most of the variability is retained on the differences within populations.

In a previous study based on mtDNA, Miranda do Douro revealed some affinities with Astur-Leonese speaking populations like Asturias and Sanabria, suggesting that the maternal diversity across the region might be in a certain extent related to language [131]. Our results do not seem to afford clues on the issue of whether such relationship indeed exists, and so the question still needs to be clarified.

Another analysis was performed using the 12 X-STRs to further explore whether any connection could be found between the genetic characteristics and the geographic proximity between the target populations. The Barrier analysis of the Zamora regions and Miranda do Douro shows three main barriers in the area: the first one isolates Miranda do Douro from all the Zamora regions; the second one discriminates Campos-Pan from Aliste and Benavente; lastly, the third one separates Sanabria from the other Zamora regions (Figure 16).

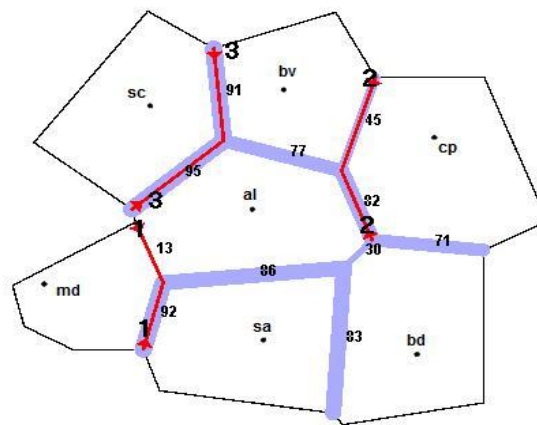


Figure 16 Three main barriers detected in the Barrier analysis. The thickness of each edge of the barrier is proportional to the number of times it was included in the computed barriers (numbers on edges).
al: Aliste; **bd:** Bajo-Duro; **bv:** Benavente; **cp:** Campos-Pan; **sa:** Sayago; **sc:** Sanabria; **md:** Miranda do Douro.

The isolation of Miranda do Douro from the Zamora regions might be a sign of the role that the Spanish-Portuguese border played as a barrier to gene flow between adjacent territories. This barrier was also detected in the study of Alvarez *et al.* (2010) [15] focusing the relationships between Zamora and its neighbour regions through the study of mtDNA. In that study the whole region of Bragança appeared separated from all Spanish areas.

Our analysis also pinpointed a separation of Sanabria from the remaining regions. The meaning of this finding is difficult to interpret since, as long as we know, there are no

demographic or historical aspects justifying the differentiation, which otherwise was not detected in the study of Alvarez *et al.* (2010) [15].

Considering the numbers represented on the edges and thickness of each barrier, it is possible to deduce a closer proximity between Miranda do Douro and the Aliste region when compared to the other barriers. Also, besides the third barrier that isolates Sanabria, the most distant region seems to be Sayago. The isolation of Sayago has also been reported by Alvarez *et al.* (2010) [15].

4.3. Comparative analysis

With the intention of obtaining a visual representation of the affinities among the populations, a Multidimensional Scaling (MDS) analysis was performed, using the corresponding pairwise F_{ST} values (Table S4 - Supplementary material). In order to include in the analysis a reasonable number of populations, the genetic distances were based on only 8 X-STR markers, for which data was available from all populations considered.

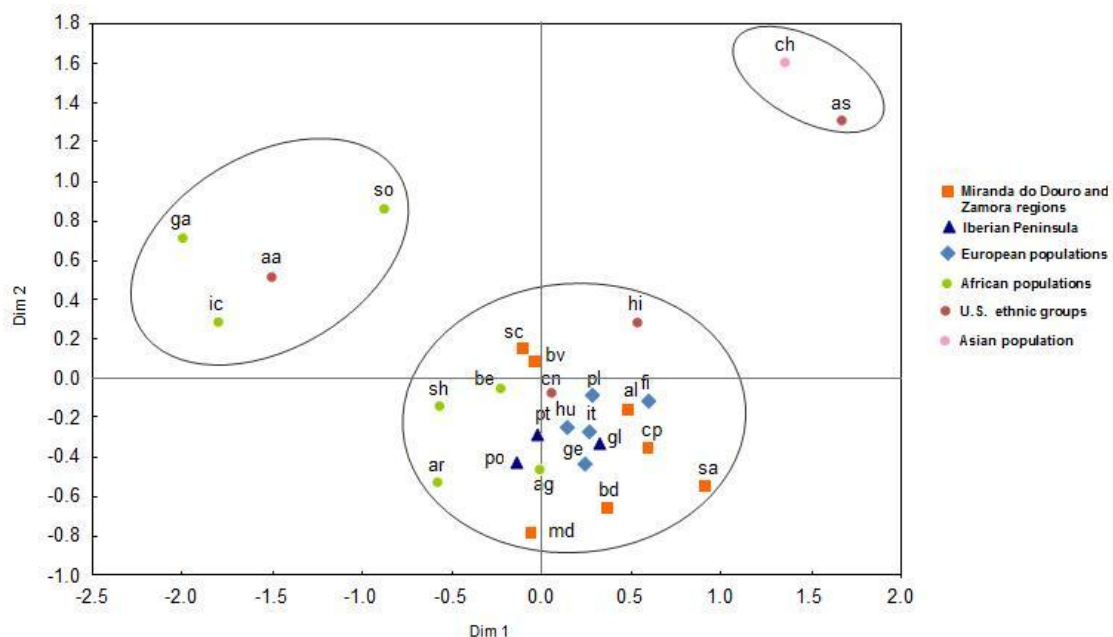


Figure 17 Plot of the Multidimensional Scaling analysis of 8 X-STRs (abbreviations in Materials and Methods section).

In the plot displayed in figure 17, there are three clear distinct groups of populations: one that is composed of samples from African populations and African-Americans, a second one with the samples from Asian populations and Asians from U.S., and a third

group made up of the samples from European and North African populations, Moroccan and Algerian. The proximity of the Moroccan, Algerian and European samples might be explained by their geographical positions, once Morocco and Algeria are North African countries, and therefore are closer to the Southern European countries, in particular, to the Iberian Peninsula. The presence, in populations of Portugal and Spain, of uniparentally transmitted lineages originated in the Northern and sub-Saharan Africa, is one of the most significant differences that the Iberian Peninsula presents compared to the rest of Europe. In part, this may be explained by the large number of migrations from Africa from the 8th century up to the 15th, including one of the most noteworthy that happened with the Muslim occupation of the Iberian Peninsula. Later on, the traffic of African slaves during the period of the mid-15th century right until the 19th century, also contributed to the introduction in Iberia of African lineages. In Portugal the demographic impact of the African slave trade was remarkable, being known for instance that around the year of 1550, 10% of the population of Lisbon was composed by African slaves [132].

However, in this plot there is not a clear pattern that shows this particular connection between Iberian Peninsula and Africa.

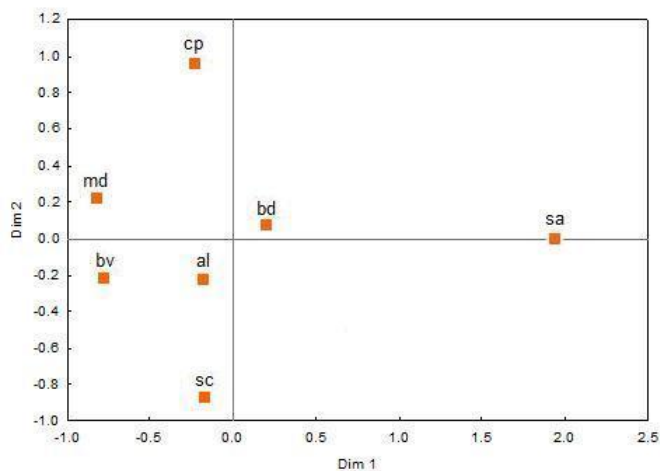


Figure 18 Plot of the Multidimensional Scaling analysis of the studied populations for 12 X-STRs, considering markers in LD.

In figure 18 is shown the MDS plot of the F_{ST} distances uniquely between the populations here studied. It is possible to observe that Miranda do Douro is well integrated within the populations from the Zamora regions. Actually Miranda occupies a position closer to five of the Zamora regions

than Sayago, which is clearly more distanced from those five neighbor Zamora regions. From the linguistic perspective, the highest affinities are shared by Miranda do Douro, Aliste and Sanabria, because the three are Leonese speaking regions. Yet, there is no evident genetic differentiation of these group populations relatively to others. This can indicate that language was not a factor stimulating connections between neighbor

regions, including the commercial and cultural connections that are well documented since long between Zamora and Miranda do Douro.

In the case of the X-Indels, a different set of countries was used for the comparisons due to the lack of published data for X-Indels. In figure 19 is presented the MDS plot obtained with the matrix of genetic distances.

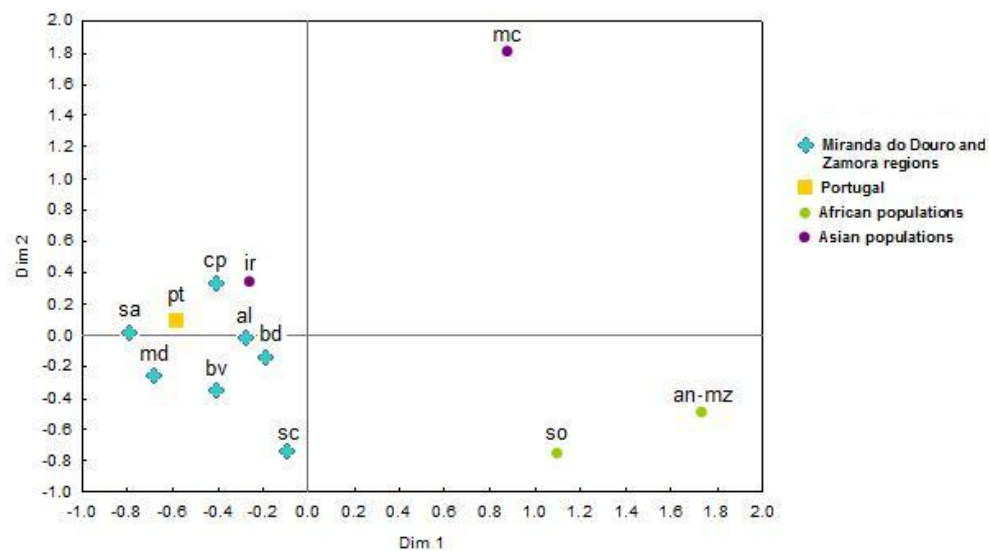


Figure 19 Plot of the Multidimensional Scaling analysis of 32 X-Indels, considering markers in LD.

Again, European populations group close together forming a cluster quite differentiated from the few representatives of African or Asian populations, which in turn occupy distinct quadrants in the plot.

Here, Miranda do Douro appears to be as close to the general sample from Portugal as to the samples from the Zamora regions.

So, the geographic proximity between Miranda do Douro and Zamora, has no reflex in their patterns of the X-Indels diversity, probably because for those markers Iberia is characterized by strong homogeneity.

5. Conclusions

In this work populations from Miranda do Douro and Zamora were studied for a set of X-chromosomal markers in order to obtain insights on the factors underlying the patterns of genetic diversity in those populations.

The results obtained with the barrier analysis, combining the X-STRs and X-Indels markers, indicate that the political frontier represents an important barrier hampering gene flow between border populations, actually isolating all the Spanish areas from the Portuguese ones, as it had been previously reported based on other genetic systems. Furthermore, some signs emerged pointing to the affinities between the populations of Miranda do Douro and Zamora, as should be expected taking into account the amount of shared cultural background and the long lasting historical and commercial connections. Even though, no significant genetic substructure was detected at the microgeographical level for the studied set of X-chromosomal markers. .

Based on the analysis of X-STRs, the population from Miranda do Douro appeared to be more closely related to that of Aliste, one of the Zamora regions, which can be a signal of the common linguistic background that the two populations have shared for centuries. Besides these two, the population from Sanabria also presents the same linguistic substrate, but yet it did not reveal any especial similarities with Miranda do Douro or Aliste.

The MDS plots of the X-STR distances showed that Miranda do Douro and Zamora are well integrated in the context of other European populations. They also revealed that Miranda presents more affinities with five of the Zamora regions, than Sayago, despite this latter region being part of Zamora as well. Considering the linguistic characteristics, it could be anticipated for the Leonese speaking regions some trend to be grouped together. However, no evidence of a genetic differentiation of these populations comparatively to the Portuguese and Castilian speaking regions was found. This puts into question a likely possibility that language has served as a motivating factor for the connection between these neighbor regions, prompting the well-known commercial and cultural ties connecting them.

In the case of the X-Indels, Miranda do Douro was actually as close to the general sample from Portugal as to the samples from the Zamora regions, falling in line with the strong Iberian homogeneity described for these markers.

Overall, our findings only provided faint hints on the relationships between Miranda and the neighbour regions from Zamora. Some results were puzzling and in most of the cases not statistically significant. The answers to the questions addressed in this study may rely in subtle features differentiating the genetic profiles of the populations, which can escape the resolution provided by the analysis of X-chromosomal markers. Possibly the study of other markers will help enlighten the issues here pursued.

Concerning levels of diversity at the X-markers, both Miranda do Douro and Zamora were highly diverse, but for most markers Miranda tended to present slightly lower diversity values than Zamora. This might represent a signature of the geographical isolation of Miranda and its small population size over the years, amplified by the more recent loss of inhabitants. However, no major signs of genetic drift were observed in Miranda, as indicates the absence of a clear reduction in diversity. In the past, Miranda often functioned as a refugee region, probably due to its remoteness, being well documented, for instance, the presence of Jewish people in the region. Furthermore, contacts with the neighbor people from the other side of the border provided the conditions for some gene influx. Both circumstances might have acted neutralizing the effect of isolation and small population size.

Comparison of the allelic distributions for the X-chromosomal markers now observed in the Miranda do Douro with previously reported data for Portuguese, led to the conclusion that Miranda does not differ from the general population of Portugal, as was demonstrated by the F_{ST} analysis, which did not reveal significant heterogeneity between Miranda and the general sample from Portugal.

So, no special attention need to be drawn to Miranda do Douro from the forensic point of view. In the field of Forensic Genetics, population substructure is an important concern. When substructure occurs, it is necessary to construct databases specific of each sub-population. Miranda do Douro does not raise that problem.

However, Zamora does need special consideration, due to its regions, particularly Sayago, since there are signs of some type of population substructure, possibly as a result of isolation.

6. References

1. Adams SM, Bosch E, Balaesque PL, Ballereau SJ, Lee AC, Arroyo E, López-Parra AM, Aler M, Grifo ASG, Brion M, Carracedo A, Lavinha J, Martínez-Jarreta B, Quintana-Murci L, Picornell A, Ramon M, Skorecki K, Behar DM, Calafell F, Jobling MA (2008) The Genetic Legacy of Religious Diversity and Intolerance: Paternal Lineages of Christians, Jews, and Muslims in the Iberian Peninsula. *The American Journal of Human Genetics* 83, 725–736
2. Cavalli-Sforza LL, Menozzi P, Piazza A (1994) *History and Geography of Human Genes*. Princeton University Press, Princeton, NJ
3. Salas A, Comas D, Laréu MV, Bertranpetit J, Carracedo (1998) A mtDNA analysis of the Galician population: a genetic edge of European variation. *European Journal of Human Genetics* 6, 365–375
4. Roewer L, Croucher PJ, Willuweit S, Lu TT, Kayser M, Lessig R, de Knijff P, Jobling MA, Tyler-Smith C, Krawczak M (2005). Signature of recent historical events in the European Y-chromosomal STR haplotype distribution. *Hum. Genet.* 116, 279–291.
5. Xiao F-X, Yotova V, Zietkiewicz E, Lovell A, Gehl D, Bourgeois S, Moreau C, Spanaki C, Plaitakis A, Moisan J-P, Labuda D (2004) Human X-chromosomal lineages in Europe reveal Middle Eastern and Asiatic contacts. *European Journal of Human Genetics* 12, 301–311
6. Novembre J, Johnson T, Bryc K, Kutalik Z, Boyko AR, Auton A, Indap A, King KS, Bergmann S, Nelson MR, Stephens M, Bustamante CD (2008) Genes mirror geography within Europe. *Nature*: November 6, 456(7218): 98–101. doi:10.1038/nature07331
7. Nelis M, Esko T, Mägi R, Zimprich F, Zimprich A, et al. (2009) Genetic Structure of Europeans: A View from the North–East. *PLoS ONE* 4(5): e5472. doi:10.1371/journal.pone.0005472
8. Barbujani G, Sokal RR (1990) Zones of sharp genetic change in Europe are also linguistic boundaries. *Proc Natl Acad Sci USA* 87:1816–1819
9. Hurler ME, Veitia R, Arroyo E, Armenteros M, Bertranpetit J, Pérez-Lezaun A, Bosch E, Shlumukova M, Cambon-Thomsen A, McElreavey K, de Munain AL, Röhl A, Wilson IJ, Singh L, Pandya A, Santos FR, Tyler-Smith C, Jobling MA (1999) Recent Male-Mediated Gene Flow over a Linguistic Barrier in Iberia, Suggested by Analysis of a Y-Chromosomal DNA Polymorphism. *Am. J. Hum. Genet.* 65:1437–1448
10. Larruga JM, Díez F, Pinto FM, Flores C, González AM (2001) Mitochondrial DNA characterisation of European isolates: The Maragatos from Spain. *European Journal of Human Genetics* 9, 708 ± 716
11. Hitti PK (1990) *The Arabs: a short history*. Gateway Editions, Washington, DC
12. Bosch E, Calafell F, Comas D, Oefner PJ, Underhill PA, Bertranpetit J (2001) High-Resolution Analysis of Human Y-Chromosome Variation Shows a Sharp Discontinuity and Limited Gene Flow between Northwestern Africa and the Iberian Peninsula *Am. J. Hum. Genet.* 68:1019–1029
13. Salvatierra V, Canto A. (2008) *Al-Ándalus de la invasión al califato de Córdoba*. Hernández E, editor. *Historia de España, Tercer Milenio*. Madrid: Síntesis. p 269.
14. Arroyo-Pardo E, Baeza C, Fernández E, López-Parra AM (2007) Genetic history of the Iberian Peninsula. Recent advances in molecular biology and evolution: applications to biological anthropology. In: Santos C, Lima M, editors. *Kerala, India: Research Signpost*. p 389–411
15. Alvarez L, Santos C, Ramos A, Pratdesaba R, Francalacci P, Aluja MP (2010) Mitochondrial DNA Patterns in the Iberian Northern Plateau: Population Dynamics and Substructure of the Zamora Province. *American Journal of Physical Anthropology* 000:000–000. doi:10.1002/ajpa.21252
16. Comissão Nacional para as Comemorações dos Descobrimentos Portugueses (1999) *Os negros em Portugal: sécs. XV a XIX*. Lisboa: Comissão Nacional para as Comemorações dos Descobrimentos Portugueses. 247p
17. Cerezo M, Achilli A, Olivieri A, Perego UA, Gómez-Carballa A, Brisighelli F, Lancioni H, Woodward SR, López-Soto M, Carracedo A, Capelli C, Torroni A, Salas A (2012) Reconstructing ancient mitochondrial DNA links between Africa and Europe. *Genome Research* doi:10.1101/gr.134452.111
18. Alvarez AG. (1983) El Dialecto Leonés: Historia y Perspectivas Futuras. *Tierras de León* 53: 81-96
19. Pidal RM (1906) El dialecto leonés. *Revista de Archivos, Bibliotecas y Museos*
20. Frias X (2001) L Mirandés: ua lhéngua minoritaria an Pertual. *Xixón : VTP ISSN 1616-413X*

21. Ferreira MB (2000) O mirandês, Língua Minoritária. Uma política de Língua para o Português, Lisboa: Colibri, 137-145
22. Vasconcelos J (1882) O dialecto mirandes. Contribução para o estudio da dialectologia romanica no dominio glotológico hispano-lusitano. Porto: Livraria Portuense
23. Vasconcelos CM (1913) Lições de filologia portuguesa. Lisboa, p.201
24. Santos MJM (1967) Os falares fronteiriços de Trás-os-Montes. Revista Portuguesa de Filologia, Coimbra: vol. XII, tomo II e vols. XIII e XIV
25. Sletsjõe L (1967) La position du mirandais. Studia Neophilologica, XXXIX:150-173
26. Mourinho A (1987) A língua mirandesa como vector cultural do Nordeste português. Actas das 1^{as} Jornadas de Língua e Cultura Mirandesas. Miranda do Douro: Câmara Municipal de Miranda do Douro, pp. 75-87
27. Cruz LS, Saramago J, Vitorino G (1994) Os falares leoneses em território português: coesão e diversidade. Actas do Encontro Regional da Associação Portuguesa de Linguística: Variação linguística no espaço, no tempo e na sociedade (Miranda do Douro, Setembro). Lisboa APL, Colibri, pp. 281-293
28. Ferreira MB (1999) Lição de mirandês "You falo cumo bós I bós nun falais cumo you". Estudios de Sociolingüística Románica. Linguas e variedades minorizadas, edition: F. Fernández Rei e A. Santamarina Fernández, Santiago de Compostela, p. 133-153
29. Mourinho A (1980) Roma na terra de Miranda. Actas do Seminário de Arqueologia do Noroeste peninsular, Guimarães
30. Sánchez-Albornoz C (1966) Despoblación y repoblación del valle del Duero. Buenos Aires
31. Carvalho JH (1952) Porqué se falam dialectos leoneses em terras de Miranda? Revista Portuguesa de Filologia 5:265-280
32. Pidal M (1959) Dos problemas iniciales relativos a los romances hispánicos. Enciclopedia lingüística hispánica. Madrid, Vol I p. XLVII
33. Alves AB (2007) Palavras de Identidade de Miranda. Uma abordagem estatístico-pragmática de contos da literatura oral mirandesa. Publicações Pena Perfeita. ISBN:978-972-8925-47-5
34. Ferreira MB (1995) O mirandês e as línguas do noroeste peninsular. Lletres Asturianas, 57, 7-22
35. Ohno S (1967) Sex chromosomes and sex-linked genes. Springer-Verlag, New York
36. Lahn BT, Page DC (1999) Four evolutionary strata on the human X chromosome. *Science* 286: 964–967
37. Lahn BT, Pearson NM, Jegalian K (2001) The human Y chromosome, in the light of evolution. *Nat. Rev. Genet.* 2: 207–216
38. Bachtrog D (2006) A dynamic view of sex chromosome evolution. *Current Opinion in Genetics & Development*, Vol 16, Issue 6, December, p578–585. doi:10.1016/j.gde.2006.10.007
39. Athanasiadis G, Esteban E, Via M, Dugoujon J-M, Moschonas N, Chaabani H, Moral P (2007) The X chromosome Alu insertions as a tool for human population genetics: data from European and African human groups. *European Journal of Human Genetics* 15, 578–583
40. Pereira R, Gomes I, Amorim A, Gusmão L (2007) Genetic diversity of 10 X chromosome STRs in northern Portugal. *Int J Legal Med* 121:192–197
41. Szibor R, Hering S, Edelmann J (2006) A new Web site compiling forensic chromosome X research is now online. *Int J Legal Med* 120:252–254
42. Lander ES, Linton LM, Birren B et al (2001) Initial sequencing and analysis of the human genome. *Nature* 409:860–921
43. Li WH, Yi S, Makova K (2002) Male-driven evolution. *Curr Opin Genet Dev* 12:650–656
44. Schaffner SF (2004) The X chromosome in population genetics. *Nat Rev Genet* 5:43–51
45. Tomas C, Pereira V, Morling N (2011) Analysis of 12 X-STRs in Greenlanders, Danes and Somalis using Argus X-12. *Int J Legal Med* doi:10.1007/s00414-011-0609-y
46. Laan M, Wiebe V, Khusnutdinova E, Remm M, Pääbo S (2004) X-chromosome as a marker for population history: linkage disequilibrium and haplotype study in Eurasian populations. *Eur J Hum Genet* 13:452–462

47. Pereira R, Pereira V, Gomes I, Tomas C, Morling N, Amorim A, Prata MJ, Carracedo A, Gusmão L (2012) A method for the analysis of 32 X chromosome insertion deletion polymorphisms in a single PCR. *Int J Legal Med.* doi:10.1007/s00414-011-0593-2
48. Ribeiro-Rodrigues EM, Santos NP, Ribeiro-dos-Santos AK, Pereira R, Amorim A, Gusmão L, Zago MA, Santos SE (2009) Assessing interethnic admixture using an X-linked insertion–deletion multiplex. *Am J Hum Biol* 21(5):707–709. doi:10.1002/ajhb.20950
49. Edelman J, Hering S, Augustin C, Szibor R (2009) Indel polymorphisms—An additional set of markers on the X-chromosome. *Forensic Sci Int Genet Supplement Series 2* (1):510–512. doi:10.1016/j.fsigss.2009.08.148
50. Casto AM, Li JZ, Absher D, Myers R, Ramachandran S, Feldman MW (2010) Characterization of X-linked SNP genotypic variation in globally distributed human populations. *Genome Biol* 11(1):R10. doi:10.1186/gb-2010-11-1-r10
51. Gomes I, Prinz M, Pereira R, Meyers C, Mikulasovich RS, Amorim A, Carracedo A, Gusmão L (2007) Genetic analysis of three US population groups using an X-chromosomal STR decaplex. *Int J Legal Med* 121(3):198–203. doi:10.1007/s00414-006-0146-2
52. Tomas C, Sanchez JJ, Barbaro A, Brandt-Casadevall C, Hernandez A, Ben Dhiab M, Ramon M, Morling N (2008) X-chromosome SNP analyses in 11 human Mediterranean populations show a high overall genetic homogeneity except in North-west Africans (Moroccans). *BMC Evol Biol* 8:75. doi:10.1186/1471-2148-8-75
53. Freitas NS, Resque RL, Ribeiro-Rodrigues EM, Guerreiro JF, Santos NP, Ribeiro-dos-Santos AK, Santos SE (2010) X-linked insertion/deletion polymorphisms: forensic applications of a 33- markers panel. *Int J Legal Med* 124(6):589–593. doi:10.1007/s00414-010-0441-9
54. Tomas C, Sanchez JJ, Castro JA, Borsting C, Morling N (2010) Forensic usefulness of a 25 X-chromosome single-nucleotide polymorphism marker set. *Transfusion (Paris)* 50(10):2258–2265. doi:10.1111/j.1537-2995.2010.02696.x
55. Bustamante CD, Ramachandran S (2009) Evaluating signatures of sex-specific processes in the human genome. *Nat Genet.* January; 41(1): 8–10. doi:10.1038/ng0109-8
56. Keinan A, Mullikin JC, Patterson N, Reich D (2009) Accelerated genetic drift on chromosome X during the human dispersal out of Africa. *Nat Genet.* January; 41(1): 66–70. doi:10.1038/ng.303
57. Labuda D, Lefebvre JF, Nadeau P, Roy-Gagnon MH (2010) Female-to-Male Breeding Ratio in Modern Humans—an Analysis Based on Historical Recombinations. *The American Journal of Human Genetics* 86, 353–363. doi:10.1016/j.ajhg.2010.01.029
58. Hammer MF, Mendez FL, Cox MP, Woerner AE, Wall JD (2008) Sex-Biased Evolutionary Forces Shape Genomic Patterns of Human Diversity. *PLoS Genetics* Vol.4 Issue 9 e1000202
59. Bedoya G, et al. (2006) Admixture dynamics in Hispanics: a shift in the nuclear genetic ancestry of a South American population isolate. *Proc Natl Acad Sci U S A* 103:7234–9. [PubMed:16648268]
60. Hammer MF, et al. (2001) Hierarchical patterns of global human Y-chromosome diversity. *Mol Biol Evol* 18:1189–203. [PubMed: 11420360]
61. Helgason A, et al. (2000) Estimating Scandinavian and Gaelic ancestry in the male settlers of Iceland. *Am J Hum Genet* 67:697–717. [PubMed: 10931763]
62. Parra EJ, et al. (1998) Estimating African American admixture proportions by use of population-specific alleles. *Am J Hum Genet* 63:1839–51. [PubMed: 9837836]
63. Kayser M, et al. (2003) Reduced Y-chromosome, but not mitochondrial DNA, diversity in human populations from West New Guinea. *Am J Hum Genet* 72:281–302. [PubMed: 12532283]
64. Seielstad MT, Minch E, Cavalli-Sforza LL. (1998) Genetic evidence for a higher female migration rate in humans. *Nat Genet* 20:278–80. [PubMed: 9806547]
65. Marlowe FW. (2005) Hunter-gatherers and human evolution. *Evolutionary Anthropology* 14:54–67
66. Ellegren H (2004) Microsatellites: Simple Sequences with Complex Evolution. *Nature Reviews Genetics* Vol.5 doi:10.1038/nrg1348
67. Becker D, Rodig H, Augustin C, Edelman J, Götz F, Hering S, Szibor R, Brabetz W (2008) Population genetic evaluation of eight X-chromosomal short tandem repeat loci using Mentype Argus X-8 PCR amplification kit. *FSI Genetics* 2:69–74
68. Szibor R (2007) X-chromosomal markers: Past, present and future. *Forensic Science International: Genetics* 1, 93–99

69. Rodig H, Kloep F, Weißbach L, Augustin C, Edelmann J, Hering S, Szibor R, Götz F, Brabetz W (2010) Evaluation of seven X-chromosomal short tandem repeat loci located within the Xq26 region. *Forensic Science International: Genetics* 4, 194–199
70. Augustin C, Cichy R, Hering S, Edelmann J, Kuhlisch E, Szibor R (2006) Forensic evaluation of three closely linked STR markers in a 13 kb region at Xp11.23, in: C. Doutremepuich (Ed.), *Progress in Forensic Genetics 11-Proceedings of the 21st International ISFG Congress*, Ponta Delgada, Portugal, September 13–17, 2005, Elsevier Science, Amsterdam, The Netherlands
71. Edwards A, Hammond HA, Jin L, Caskey CT, Chakraborty R (1992) Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups, *Genomics* 12, 241–253
72. Kishida T, Wang W, Fukuda M, Tamaki Y (1997) Duplex PCR of the Y-27H39 and HPRT loci with reference to Japanese population data on the HPRT locus, *Jpn. J. Legal Med.* 51, 67–69
73. Ribeiro-Rodrigues EM, Palha TJB, Bittencourt EA, Ribeiro-dos-Santos A, Santos S (2011) Extensive survey of 12 X-STRs reveals genetic heterogeneity among Brazilian populations. *Int J Legal Med* (2011) 125:445–452 doi:10.1007/s00414-011-0561-x
74. Bekada A, Benhamamouch S, Boudjema A, Fodil M, Menegon S, Torre C, Robino C (2009) Analysis of 12 X-chromosomal STRs in an Algerian population sample. *Forensic Sci Int Genet Suppl Ser* 2:400–401
75. Tamura A, Tsutsumi H, Hara M, Takada A, Saito K, Suzuki K, Komuro T (2009) Genetic studies of eight X-STRs in a Japanese population. *Leg Med* 11:S451–S452
76. Gusmão L, Sánchez-Diz P, Alves C, Gomes I, Zarrabeitia MT, Abovich M, Atmetlla I, Bobillo C, Bravo L, Builes J, Cainé L, Calvo R, Carvalho E, Carvalho M, Cicarelli R, Catelli L, Corach D, Espinoza M, García O, Malaghini M, Martins J, Pinheiro F, Porto MJ, Raimondi E, Riancho JÁ, Rodríguez A, Rodríguez A, Cardozo AR, Schneider V, Silva S, Tavares C, Toscanini U, Vullo C, Whittle M, Yurrebaso I, Carracedo A, Amorim A (2009) A GEP-ISFG collaborative study on the optimization of an X-STR decaplex: data on 15 Iberian and Latin American populations. *Int J Leg Med* 123:227–234
77. Leite FPN, Santos SEB, Rodríguez EMR, Callegari-Jacques SM, Demarchi DA, Tsuneto LT, Petzl-Erler ML, Salzano FM, Hutz MH (2009) Linkage disequilibrium patterns and genetic structure of Amerindian and non-Amerindian Brazilian populations revealed by long-range X-STR markers. *Am J Phys Anthropol* 139:404–412
78. Gomes I, Prinz M, Pereira R, Meyers C, Mikulasovich RS, Amorim A, Carracedo A, Gusmão L (2007) Genetic analysis of three US population groups using an X-chromosomal STR decaplex. *Int J Legal Med* 121(3):198–203
79. Becker D, Rodig H, Augustin C, Edelmann J, Götz F, Hering S, Szibor R, Brabetz W (2008) Population genetic evaluation of eight X-chromosomal short tandem repeat loci using Mentype Argus X-8 PCR amplification kit. *FSI Genetics* 2:69–74
80. Poetsch M, Petersmann H, Repenning A, Lignitz E (2005) Development of two pentaplex systems with X-chromosomal STR loci and their allele frequencies in a northeast German population. *Forensic Sci Int* 155:71–76
81. Hering S, Augustin C, Edelmann J, Heide M, Dressler J, Rodig H, Kuhlisch E, Szibor R (2006) DXS10079, DXS10074 and DXS10075 are STRs located within a 280-kb region of Xq12 and provide stable haplotypes useful for complex kinship cases. *Int J Legal Med* 120:337–345
82. Hundertmark T, Hering S, Edelmann J, Augustin C, Plate I, Szibor R (2008) The STR cluster DXS10148–DXS8378–DXS10135 provides a powerful tool for X-chromosomal haplotyping at Xp22. *Int J Legal Med* 122:489–495
83. Edelmann J, Hering S, Augustin C, Szibor R (2008) Characterisation of the STR markers DXS10146, DXS10134 and DXS10147 located within a 79.1 kb region at Xq28. *FSI Genetics* 2:41–46
84. Szibor R, Krawczak M, Hering S, Edelmann J, Kuhlisch E, Kraune D (2003) Use of X-linked markers for forensic purposes. *Int J Legal Med* 117:67–74
85. Tillmar AO, Mostad P, Egeland T, Lindblom B, Holmlund G, Montelius K (2008) Analysis of linkage and linkage disequilibrium for eight X-STR markers. *Forensic Science International: Genetics* 3 37–41
86. Ott J (1999) *Analysis of Human Genetic Linkage*, 3rd. ed., The Johns Hopkins University Press, Baltimore.
87. Amorim CEG, Wang S, Marrero AR, Salzano FM, Ruiz-Linares A, Bortolini MC (2011) X-Chromosomal Genetic Diversity and Linkage Disequilibrium Patterns in Amerindians and Non-Amerindian Populations. *American Journal of Human Biology* 23:299–304

88. Branco CC, Cabrol E, Bento MS, Gomes CT, Cabral R, Vicente AM, Pacheco PR, Mota-Vieira L (2008) Evaluation of Linkage Disequilibrium on the Xq13.3 Region: Comparison Between the Azores Islands and Mainland Portugal. *American Journal of Human Biology* 20:364–366
89. Zarrabeitia MT, Pinheiro F, de Pancorbo MM, Cainé L, Cardoso S, Gusmão L, Riancho JA (2009) Analysis of 10 X-linked tetranucleotide markers in mixed and isolated populations. *Forensic Science International: Genetics* 3, 63-66
90. Mills RE, Luttig CT, Larkins CE, Beauchamp A, Tsui C, Pittard WS, Devine SE (2006) An initial map of insertion and deletion (INDEL) variation in the human genome. Cold Spring Harbor Laboratory Press 16:1182–1190
91. Dawson E, Chen Y, Hunt S, Smink LJ, Hunt A, Rice K, Livingston S, Bumpstead S, Bruskiewich R, Sham P, et al. (2001) A SNP resource for human chromosome 22: Extracting dense clusters of SNPs from the genomic sequence. *Genome Res.* 11: 170–178
92. Sachidanandam R, Weissman D, Schmidt SC, Kakol JM, Stein LD, Marth G, Sherry S, Mullikin JC, Mortimore BJ, Willey DL, Hunt SE, Cole CG, Coggill PC, Rice CM, Ning Z, Rogers J, Bentley DR, Kwok PY, Mardis ER, Yeh RT, Schultz B, Cook L, Davenport R, Dante M, Fulton L, Hillier L, Waterston RH, McPherson JD, Gilman B, Schaffner S, Van Etten WJ, Reich D, Higgins J, Daly MJ, Blumenstiel B, Baldwin J, Stange-Thomann N, Zody MC, Linton L, Lander ES, Altshuler D (2001) A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature* 409(6822):928–933. doi:10.1038/35057149
93. Weber JL, David D, Heil J, Fan Y, Zhao C, Marth G (2002) Human diallelic insertion/deletion polymorphisms. *Am J Hum Genet* 71(4):854–862. doi:10.1086/342727
94. Britten RJ, Rowen L, Williams J, Cameron RA (2003) Majority of divergence between closely related DNA samples is due to indels. *PNAS* Vol. 100, no. 8, 4661–4665
95. Lupski JR, Roth JR, Weinstock GM (1996) Chromosomal duplications in bacteria, fruit flies, and humans. *Am J Hum Genet* 58:21–27
96. Pereira R, Phillips C, Alves C, Amorim A, Carracedo A, Gusmão L (2009) A new multiplex for human identification using insertion/deletion polymorphisms. *Electrophoresis* 30(21):3682–3690. doi:10.1002/elps.200900274
97. Yang N, Li H, Criswell LA, Gregersen PK, Alarcon-Riquelme ME, Kittles R, Shigeta R (2005) Examination of ancestry and ethnic affiliation using highly informative diallelic DNA markers: application to diverse and admixed populations and implications for clinical epidemiology and forensic medicine. *Hum. Genet.*, 118, 382–392
98. Rosenberg NA, Mahajan S, Ramachandran S, Zhao C, Pritchard JK, Feldman MW (2005) Clines, Clusters, and the Effect of Study Design on the Inference of Human Population Structure. *PLoS Genet.*, 1, 660–671
99. Bastos-Rodrigues L, Pimenta JR, Pena SD (2006) The Genetic Structure of Human Populations Studied Through Short Insertion-Deletion Polymorphisms. *Ann. Hum. Genet.* 2006, 70, 658–665
100. Lareu MV, Phillips CP, Carracedo A, Lincoln AJ, Syndercombe Court D, Thomson JA (1994) Investigation of the STR locus HUMTH01 using PCR and electrophoresis formats: UK and Galician Caucasian population surveys and usefulness in paternity investigations. *Forensics Science International* 66, 41-52
101. Sambrook J, Fritsch EF, and Maniatis TE (1982) Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory Press New York
102. Mentype Argus X-12 pdf:
http://www.biotype.de/fileadmin/user/Flyer/Mentype_ArgusX-12.pdf
103. Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour* 10(3):564–567. doi:10.1111/j.1755-0998.2010.02847.x
104. Nei M (1987) Molecular evolutionary genetics. New York: Columbia University Press
105. Tajima F (1983) Evolutionary relationship of DNA sequences in finite populations. *Genetics* 105:437–460
106. Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* 48:361-372
107. Slatkin M (1994) Linkage disequilibrium in growing and stable populations. *Genetics* 137:331-336
108. Bonferroni CE (1936) Teoria statistica delle classi e calcolo delle probabilità: Pubblicazioni del R Istituto Superiore di Scienze Economiche e Commerciali di Firenze

109. Reynolds J, Weir BS, Cockerham CC (1983) Estimation for the coancestry coefficient: basis for a short-term genetic distance. *Genetics* 105:767-779
110. Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. *Genetics* 139: 457-462
111. Takezaki N, Nei M, Tamura K (2010) POPTREE2: software for constructing population trees from allele frequency data and computing other population statistics with Windows-interface. *Mol Biol Evol* 27:747–752
112. Cainé L, Costa S, Pinheiro MF (2012) Population data of 12 X-STR loci in a North of Portugal sample. *Int J Legal Med*. doi:10.1007/s00414-012-0672-z
113. Besada MG, Ferreira S, Magariños MG, Gusmão L, Diz PS (2012) Genetic characterization of Western Iberia using Mentype® Argus X-8 kit . *Forensic Science International: Genetics* 6, e39–e41. doi:10.1016/j.fsigen.2011.02.008
114. Inturri S, Menegon S, Amoroso A, Torre C, Robino C (2011) Linkage and linkage disequilibrium analysis of X-STRs in Italian families. *Forensic Science International: Genetics* 5, 152–154. doi:10.1016/j.fsigen.2010.10.012
115. Zalán A, Völgyi A, Jung M, Peterman O, Pamjav H (2005) Hungarian population data of four X-linked markers: DXS8378, DXS7132, HPRTB, and DXS7423. *Int J Legal Med*, 121: 74-77. doi:10.1007/s00414-005-0051-0
116. Zalán A, Völgyi A, Brabetz W, Schleinitz D, Pamjav H (2008) Hungarian population data of eight X-linked markers in four linkage groups. *Forensic Science International* 175, 73–78. doi:10.1016/j.forsciint.2007.05.012
117. Łuczak S, Rogalla U, Malyarchuk BA, Grzybowski T (2011) Diversity of 15 human X chromosome microsatellite loci in Polish population. *Forensic Science International: Genetics* 5, e71–e77. doi:10.1016/j.fsigen.2010.12.009
118. Hedman M, Palo JU, Sajantila A (2009) X-STR diversity patterns in the Finnish and the Somali population. *Forensic Science International: Genetics* 3, 173–178. doi:10.1016/j.fsigen.2009.02.005
119. Edelman J, Lutz-Bonengel S, Hering S (2012) X-chromosomal haplotype frequencies of four linkage groups using the Investigator Argus X-12 Kit. *Forensic Science International: Genetics* 6, e24–e34. doi:10.1016/j.fsigen.2011.01.001
120. Thiele K, Löffler S, Löffler J, Günthner F, Nitschke K, Edelmann J, Lessig R (2008) Population data of eight X-chromosomal STR markers in Ewe individuals from Ghana. *Forensic Science International: Genetics Supplement Series* 1, 167–169. doi:10.1016/j.fsigss.2007.10.059
121. Bentayebi K, Picornell A, Bouabdeallah M, Castro JA, Aboukhalid R, Squalli D, Misericordia M, Amzazi S (2012) Genetic diversity of 12 X-chromosomal short tandem repeats in the Moroccan population. *Forensic Science International: Genetics* 6 (2012) e48–e49. doi:10.1016/j.fsigen.2011.03.008
122. Pasino S, Caratti S, Del Pero M, Santovito A, Torre C, Robino C (2011) Allele and haplotype diversity of X-chromosomal STRs in Ivory Coast. *Int J Legal Med*, 125:749–752. doi:10.1007/s00414-011-0591-4
123. Diegoli T, Linacre A, Vallone PM, Butler JM, Coble MD (2011) Allele frequency distribution of twelve X-chromosomal short tandem repeat markers in four U.S. population groups. *Forensic Science International: Genetics Supplement Series* 3, e481–e483. doi:10.1016/j.fsigss.2011.09.102
124. Zeng X, Ren Z, Chen J, Lv D, Tong D, Chen H, Sun H (2011) Genetic polymorphisms of twelve X-chromosomal STR loci in Chinese Han population from Guangdong Province. *Forensic Science International: Genetics Vol 5, Issue 4*, Pages e114-e116. doi:10.1016/j.fsigen.2011.03.005
125. Pereira V, Moncada E, Diez IE, Tomas C, Amorim A, Morling N, Gusmão L, Prata MJ (2011) Genetic characterization of Somali and Iraqi populations using a set of 33 X-chromosome Indels. *Forensic Science International: Genetics Supplement Series* 3, e137–e138. doi:10.1016/j.fsigss.2011.08.069
126. Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* 131:479-491
127. Manni F, Guérard E, Heyer E (2004). Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by "Monmonier's algorithm". *Human Biology*, 76(2): 173-190

- 128.** Huel RLM, Bašić L, Madacki-Todorović K, Smajlović L, Eminović I, Berbić I, Miloš A, Parsons TJ (2007) Variant alleles, triallelic patterns, and point mutations observed in nuclear short tandem repeat typing of populations in Bosnia and Serbia. *Croat Med J.* 2007;48:494-502
- 129.** Freitas NS, Resque RL, Ribeiro-Rodrigues EM, Guerreiro JF, Santos NP, Ribeiro-dos-Santos A, Santos S (2010) X-linked insertion/deletion polymorphisms: forensic applications of a 33-markers panel. *Int J Legal Med* 124:589–593
- 130.** Matisse TC, Chen Fang, Chen W *et al.* (2007) A second-generation combined linkage physical map of the human genome. *Genome Res* 17:1783-6
- 131.** Cãnovas QM (2011) Patrón de linajes femeninos en la población de habla mirandesa. Masters thesis – not published
- 132.** Pereira L, Prata MJ, and Amorim A (2000) Diversity of mtDNA lineages in Portugal: not a genetic edge of European variation. *Ann Hum Genet* 64(Pt 6):491-506

7. Supplementary Material

Table S1 Genetic profiles obtained with the amplification of the 12 X-STRs.

			Linkage Group 1			Linkage Group 2			Linkage Group 3			Linkage Group 4		
Sample	AM		DXS10148	DXS10135	DXS8378	DXS7132	DXS10079	DXS10074	DXS10103	HPRTB	DXS10101	DXS10146	DXS10134	DXS7423
Miranda do Douro	MD 02	XY	25.1	24	11	14	20	8	20	11	27.2	41.2	38.3	15
	MD 07	XY	28.1	20	12	14	21	18	17	12	32	28	34	15
	MD 09	XY	23	23	12	13	22	18	19	12	30.2	30	35	14
	MD 11	XY	18	28	12	14	21	16	19	8	32	28	40.3	15
	MD 19	XY	27.1	20	10	13	15	17	18	13	29	29	36	15
	MD 25	XY	20.1	33	11	13	19	18	19	13	28.2	28	33	16
	MD 50	XY	18	29	11	15	20	14	19	13	30.2	43.2	43.3	15
	MD 51	XY	24	24	10	14	19	16	18	12	31.2	29	34	14
	MD 58	XY	24.1	18	10	14	21	17	19	12	31.2	27	40.3	14
	MD 59	XY	26.1	28	12	13	20	16	21	14	28.2	25	37.3	15
	MD 61	XY	23.1	25	12	15	20	17	19	11	27.2	29	35	15
	MD 64	XY	27.1	17	11	13	21	17	17	14	32	28	38	13
	MD 73	XY	20.1	33	11	14	22	7	17	12	32	40.2	41.3	16
	MD 93	XY	23	33	12	12	20	14	20	11	30.2	38.2	39	16
	MD 94	XY	23.1	22	12	14	19	17	19	13	30	29	37	13
	MD L05	XY	25.1	20	10	13	17	8	19	11	27.2	29	37	14
	MD L09	XY	27.1	23	11	14	20	19	20	13	28.2	29	35	14
	MD L11	XY	25.1	18	12	15	21	17	20	14	30.2	28	35	15
	MD L12	XY	18	28	12	13	18	8	18	11	27.2	28	35	14
	MD L22	XY	25.1	28	10	15	18	16	20	13	30.2	29	35	15
	MD L25	XY	24	19	10	13	16	15	19	13	30.2	28	37	16
	MD L26	XY	27.1	23	10	14	19	7	15	12	31	39.2	37	15
	MD L29	XY	20.1	33	11	12	19	16	19	13	27.2	28	37	16
	MD L32	XY	25.1	21.1	10	13	20	7	18	12	29.2	27	38	14
	MD L34	XY	18	19.1	10	12	16	16	16	12	28.2	29	36	14
	MD L35	XY	30.1	23	11	13	20	7	19	12	29	26	35	14
	MD L38	XY	27.1	21	10	15	22	15	19	13	33.2	28	37	15

MD L39	XY	22.1	28	11	14	22	18	20	14	32	41.2	40.3	15
MD L46	XY	18	20	10	12	20	8	19	13	31.2	28	37	15
MD L49	XY	28.1	24	12	15	15	18	18	11	29.2	29	37	14
MD L56	XY	27.1	31	10	15	15	18	19	14	32.2	40.2	41.3	14
MD L57	XY	25.1	28	10	13	17	8	19	11	28.2	45.2	40.3	14
MD L60	XY	?	27	11	13	18	8	19	12	29	29	39.3	15
MD S1	XY	23	26	11	15	20	16	19	12	28	29	40.3	14
MD S2	XY	26.1	23	11	14	19	16	19	13	30.2	26	35	15
MD S5	XY	28.1	29	10	14	19	18	17	12	29.2	30	35	15
MD S7	XY	23.1	22	10	13	19	18	19	13	28	27	37	14
MD S8	XY	24.1	22	11	13	21	15	18	12	30.2	31	36	14
MD S9	XY	23.1	20	12	13	20	18	19	12	28	28	36	15
MD S10	XY	25.1	21.1	10	15	21	18	15	13	31	26	39.3	16
MD S13	XY	23.1	25	11	15	20	16	19	12	29	29	35	14
MD S16	XY	29.1	25	11	13	15	17	20	12	32.2	43.2	40.3	14
MD S24	XY	27.1	18	10	13	18	15	18	12	31.2	41.2	32	15
MD S27	XY	27.1	19.1	12	12	19	19	16	16	33	26	36	14
MD S30	XY	30.1	23	11	12	18	14	19	13	31.2	27	36	14
MD S31	XY	27.1	17	11	13	19	15	18	12	27.2	28	35	13
MD S32	XY	28	17	11	14	20	18	19	12	28.2	29	35	14
MD S33	XY	28.1	28	11	14	20	16	16	13	29.2	29	36	14
MD S34	XY	27.1	17	11	13	21	15	19	13	28.2	31	34	16
MD S35	XY	25.1	21.1	10	15	18	17	19	12	28.2	26	38	15
MD S36	XY	25.1	20	12	13	17	8	16	13	31	26	38	15
MD X2	XY	23	23.1	10	14	16	16	19	12	30.2	39.2	35	15
MD X3	XY	27.1	23	13	14	20	17	19	11	27.2	29	35	15
MD X6	XY	18	19.1	12	14	17	8	18	13	30.2	44.2	39.3	14
MD X7	XY	18	28	12	16	20	18	19	13	32	30	36	15
MD 4	XX	25.1	24	11	14	20	8	20	11	27.2	41.2	38.3	15
MD 02	XY	28.1	20	12	14	21	18	17	12	32	28	34	15
MD 07	XY	23	23	12	13	22	18	19	12	30.2	30	35	14

MD 09	XY	18	28	12	14	21	16	19	8	32	28	40.3	15
MD 11	XY	27.1	20	10	13	15	17	18	13	29	29	36	15
MD 19	XY	20.1	33	11	13	19	18	19	13	28.2	28	33	16
MD 25	XY	18	29	11	15	20	14	19	13	30.2	43.2	43.3	15
MD 50	XY	24	24	10	14	19	16	18	12	31.2	29	34	14
MD 51	XY	24.1	18	10	14	21	17	19	12	31.2	27	40.3	14
MD 58	XY	26.1	28	12	13	20	16	21	14	28.2	25	37.3	15
MD 59	XY	23.1	25	12	15	20	17	19	11	27.2	29	35	15
MD 61	XY	27.1	17	11	13	21	17	17	14	32	28	38	13
MD 64	XY	20.1	33	11	14	22	7	17	12	32	40.2	41.3	16
MD 73	XY	23	33	12	12	20	14	20	11	30.2	38.2	39	16
MD 93	XY	23.1	22	12	14	19	17	19	13	30	29	37	13
MD 94	XY	25.1	20	10	13	17	8	19	11	27.2	29	37	14
MD L05	XY	27.1	23	11	14	20	19	20	13	28.2	29	35	14
MD L09	XY	25.1	18	12	15	21	17	20	14	30.2	28	35	15
MD L11	XY	18	28	12	13	18	8	18	11	27.2	28	35	14
MD L12	XY	25.1	28	10	15	18	16	20	13	30.2	29	35	15
MD L22	XY	24	19	10	13	16	15	19	13	30.2	28	37	16
MD L25	XY	27.1	23	10	14	19	7	15	12	31	39.2	37	15
MD L26	XY	20.1	33	11	12	19	16	19	13	27.2	28	37	16
MD L29	XY	25.1	21.1	10	13	20	7	18	12	29.2	27	38	14
MD L32	XY	18	19.1	10	12	16	16	16	12	28.2	29	36	14
MD L34	XY	30.1	23	11	13	20	7	19	12	29	26	35	14
MD L35	XY	27.1	21	10	15	22	15	19	13	33.2	28	37	15
MD L38	XY	22.1	28	11	14	22	18	20	14	32	41.2	40.3	15
MD L39	XY	18	20	10	12	20	8	19	13	31.2	28	37	15
MD L46	XY	28.1	24	12	15	15	18	18	11	29.2	29	37	14
MD L49	XY	27.1	31	10	15	15	18	19	14	32.2	40.2	41.3	14
MD L56	XY	25.1	28	10	13	17	8	19	11	28.2	45.2	40.3	14
MD L57	XY	?	27	11	13	18	8	19	12	29	29	39.3	15
MD L60	XY	23	26	11	15	20	16	19	12	28	29	40.3	14

MD S1	XY	26.1	23	11	14	19	16	19	13	30.2	26	35	15
MD S2	XY	28.1	29	10	14	19	18	17	12	29.2	30	35	15
MD S5	XY	23.1	22	10	13	19	18	19	13	28	27	37	14
MD S7	XY	24.1	22	11	13	21	15	18	12	30.2	31	36	14
MD S8	XY	23.1	20	12	13	20	18	19	12	28	28	36	15
MD S9	XY	25.1	21.1	10	15	21	18	15	13	31	26	39.3	16
MD S10	XY	23.1	25	11	15	20	16	19	12	29	29	35	14
MD S13	XY	29.1	25	11	13	15	17	20	12	32.2	43.2	40.3	14
MD S16	XY	27.1	18	10	13	18	15	18	12	31.2	41.2	32	15
MD S24	XY	27.1	19.1	12	12	19	19	16	16	33	26	36	14
MD S27	XY	30.1	23	11	12	18	14	19	13	31.2	27	36	14
MD S30	XY	27.1	17	11	13	19	15	18	12	27.2	28	35	13
MD S31	XY	28	17	11	14	20	18	19	12	28.2	29	35	14
MD S32	XY	28.1	28	11	14	20	16	16	13	29.2	29	36	14
MD S33	XY	27.1	17	11	13	21	15	19	13	28.2	31	34	16
MD S34	XY	25.1	21.1	10	15	18	17	19	12	28.2	26	38	15
MD S35	XY	25.1	20	12	13	17	8	16	13	31	26	38	15
MD S36	XY	23	23.1	10	14	16	16	19	12	30.2	39.2	35	15
MD X2	XY	27.1	23	13	14	20	17	19	11	27.2	29	35	15
MD X3	XY	18	19.1	12	14	17	8	18	13	30.2	44.2	39.3	14
MD X6	XY	18	28	12	16	20	18	19	13	32	30	36	15
MD X7	XY	25.1	24	11	14	20	8	20	11	27.2	41.2	38.3	15
MD 4	XX	22.1 - 24.1	20.1 - 27	10 - 10	14 - 15	19 - 20	16 - 18	19 - 19	12 - 12	31.2 - 31.2	28 - 28	35 - 36	13 - 14
MD 5	XX	18 - 28.1	30 - 30	10 - 12	13 - 13	18 - 19	15 - 17	18 - 19	11 - 11	27.2 - 31.2	39.2 - 42.2	34 - 35	13 - 15
MD 6	XX	23.1 - 24.1	22 - 24	10 - 11	12 - 13	19 - 20	18 - 18	19 - 19	13 - 13	28 - 32.2	27 - 27	36 - 37	14 - 15
MD 8	XX	22.1 - 29.1	18 - 26	11 - 13	12 - 14	20 - 20	8 - 18	19 - 19	12 - 13	31.2 - 32.2	29 - 43.2	36 - 40.3	14 - 14
MD 10	XX	23.1 - 28.1	22 - 24	10 - 10	14 - 16	16 - 20	16 - 16	19 - 19	12 - 14	28 - 32	29 - 39.2	38 - 39.3	14 - 15
MD 12	XX	18 - 25.1	24 - 29	11 - 11	12 - 13	18 - 19	7 - 8	19 - 20	11 - 13	27.2 - 29.2	26 - 27	35 - 36	15 - 16
MD 15	XX	20.1 - 23	26 - 33	10 - 11	13 - 17	18 - 20	8 - 16	18 - 19	12 - 13	28 - 33	26 - 29	34 - 36	15 - 16
MD 17	XX	19 - 23	22 - 23	10 - 11	13 - 14	18 - 20	17 - 17	17 - 18	12 - 13	28.2 - 32	27 - 40.2	38 - 39.3	14 - 14
MD 18	XX	18 - 26.1	22 - 22	10 - 11	14 - 15	19 - 20	8 - 17	16 - 18	12 - 12	28.2 - 29.2	28 - 44.2	35 - 35	15 - 16

MD 20	XX	18 - 24.1	26 - 28	12 - 12	12 - 15	17 - 20	9 - 16	19 - 20	12 - 13	28 - 33.2	27 - 47.2	34 - 38	14 - 15
MD 21	XX	18 - 29.1	20 - 25	10 - 11	13 - 14	19 - 23	7 - 18	19 - 19	12 - 13	28 - 31.2	27 - 28	37 - 38	14 - 16
MD 26	XX	18 - 18	20 - 20.1	10 - 10	13 - 13	19 - 21	13 - 18	19 - 20	12 - 13	29 - 30.2	26 - 41.2	35 - 35	15 - 15
MD 27	XX	18 - 26.1	24 - 27	12 - 12	13 - 13	17 - 20	8 - 18	18 - 20	13 - 15	29 - 31.2	27 - 29	35 - 37.3	16 - 16
MD 48	XX	24.1 - 24.1	20 - 33	12 - 12	13 - 14	17 - 19	8 - 17	18 - 19	11 - 12	29.2 - 30.2	29 - 44.2	39.3 - 40.3	14 - 14
MD 54	XX	26.1 - 27.1	23 - 23.1	11 - 12	13 - 13	18 - 19	17 - 18	19 - 19	12 - 13	31 - 31.2	27 - 42.2	33 - 37	14 - 15
MD 55	XX	27.1 - 28.1	23.1 - 25	11 - 11	13 - 14	18 - 19	17 - 17	18 - 19	12 - 13	30 - 31	27 - 42.2	33 - 36	15 - 15
MD 56	XX	18 - 28.1	19 - 28	11 - 13	14 - 14	16 - 20	15 - 18	18 - 19	12 - 15	29 - 30.2	29 - 30	34 - 38	14 - 14
MD 57	XX	22.1 - 23	22 - 23.1	10 - 10	14 - 16	20 - 20	15 - 16	16 - 19	12 - 14	29.2 - 30.2	29 - 41.2	38 - 39.3	14 - 15
MD 60	XX	26.1 - 27.1	19.1 - 26	12 - 12	13 - 13	20 - 20	18 - 18	20 - 20	13 - 14	30 - 30.2	29 - 44.2	34 - 34	14 - 16
MD 62	XX	26.1 - 27.1	22 - 34	11 - 12	15 - 15	15 - 22	16 - 18	18 - 18	13 - 13	30 - 30.2	26 - 28	35 - 37	15 - 15
MD 63	XX	28.1 - 28.1	19 - 29	10 - 11	14 - 14	18 - 19	8 - 18	18 - 18	11 - 15	29 - 30.2	27 - 29	33 - 35	14 - 15
MD 65	XX	17 - 27.1	21 - 22.1	10 - 10	11 - 13	19 - 21	8 - 17	17 - 17	14 - 15	29 - 32	28 - 29	37 - 40.3	15 - 16
MD 66	XX	26.1 - 27.1	17 - 27	11 - 12	13 - 15	19 - 20	17 - 17	15 - 19	11 - 13	30.2 - 30.2	29 - 40.2	35 - 38	15 - 15
MD 67	XX	18 - 26.1	20 - 24	10 - 10	13 - 13	19 - 21	17 - 18	19 - 20	11 - 13	29.2 - 30.2	29 - 41.2	35 - 40.3	14 - 15
MD 69	XX	25.1 - 25.1	21 - 29	10 - 11	14 - 14	17 - 21	8 - 16	18 - 19	11 - 12	28.2 - 30	27 - 30	35 - 37.3	14 - 16
MD 70	XX	19 - 28.1	21 - 22	11 - 12	13 - 14	20 - 21	15 - 17	17 - 19	11 - 13	28.2 - 32	27 - 28	37 - 37.3	15 - 16
MD 71	XX	18 - 30.1	23 - 31	10 - 11	11 - 13	20 - 20	16 - 17	19 - 19	12 - 14	28 - 31.2	26 - 43.2	36 - 37	13 - 14
MD 72	XX	20 - 28.1	19 - 21.1	10 - 12	11 - 13	20 - 21	7 - 16	19 - 19	12 - 13	28 - 29.2	25 - 30	36 - 37	14 - 14
MD 74	XX	19 - 23	23 - 29	10 - 12	13 - 14	20 - 22	7 - 8	16 - 19	12 - 14	30 - 32	28 - 31	37 - 37	14 - 14
MD 89	XX	20.1 - 26.1	21 - 33	10 - 11	12 - 14	15 - 19	16 - 18	19 - 19	13 - 13	27.2 - 31.2	28 - 28	37 - 40.3	14 - 16
MD 90	XX	25.1 - 27.1	22 - 31	10 - 11	13 - 14	19 - 20	17 - 17	18 - 19	12 - 13	30 - 31.2	40.2 - 40.2	35 - 42.3	15 - 17
MD L01	XX	18 - 26.1	22 - 26	11 - 11	14 - 15	18 - 22	8 - 16	18 - 20	13 - 14	30 - 32	28 - 29	33 - 37	14 - 15
MD L03	XX	18 - 28.1	19 - 28	11 - 13	14 - 14	16 - 20	15 - 18	18 - 19	12 - 15	29 - 30.2	29 - 30	34 - 38	14 - 14
MD L06	XX	24.1 - 25.1	18 - 26	11 - 12	13 - 13	18 - 18	8 - 17	19 - 20	12 - 12	28 - 30.2	22 - 29	37 - 38	14 - 15
MD L08	XX	22.1 - 25.1	23 - 26	11 - 12	13 - 15	15 - 19	8 - 18	18 - 19	13 - 13	30.2 - 30.2	27 - 29	37 - 40.3	14 - 14
MD L10	XX	25.1 - 27.1	30 - 34	10 - 12	14 - 15	17 - 18	8 - 16	19 - 19	11 - 13	30.2 - 31.2	26 - 30	35 - 37.3	14 - 15
MD L13	XX	24.1 - 27.1	18 - 33	10 - 12	13 - 13	19 - 19	8 - 16	18 - 18	12 - 13	30 - 30.2	26 - 28	33 - 36	14 - 14
MD L14	XX	26.1 - 27.1	24 - 27	10 - 11	13 - 15	18 - 20	16 - 17	18 - 19	13 - 14	30.2 - 30.2	29 - 30	35 - 36	14 - 15
MD L15	XX	25 - 25	21 - 23	10 - 12	13 - 15	20 - 21	17 - 20	18 - 19	12 - 13	28 - 30	29 - 44.2	34 - 35	15 - 16
MD L16	XX	26.1 - 28.1	21 - 29	10 - 12	13 - 15	18 - 19	15 - 17	18 - 18	12 - 12	30.2 - 31	27 - 29	34 - 36	14 - 15

	MD L17	XX	22.1 - 28.1	24 - 26	10 - 12	15 - 16	19 - 20	15 - 17	19 - 20	13 - 14	30 - 30.2	27 - 31	34 - 38	13 - 14
	MD L20	XX	26.1 - 27.1	19 - 22	11 - 13	13 - 15	19 - 20	7 - 16	18 - 19	13 - 15	30 - 33	29 - 40.2	35 - 41.3	14 - 16
	MD L23	XX	26.1 - 26.1	22 - 22	12 - 12	13 - 14	15 - 22	7 - 15	18 - 20	13 - 13	28.2 - 30	25 - 28	35 - 36	15 - 16
	MD L28	XX	25.1 - 28.1	25 - 29	10 - 12	13 - 15	18 - 19	17 - 18	19 - 19	12 - 13	28.2 - 32	42.2 - 45.2	34 - 39	15 - 15
	MD L36	XX	20.1 - 24.1	19 - 33	11 - 12	12 - 15	15 - 20	8 - 18	19 - 19	12 - 12	27.2 - 30.2	29 - 29	40.3 - 43.3	14 - 14
	MD L37	XX	28.1 - 30.1	23 - 30	10 - 11	13 - 15	20 - 22	7 - 16	19 - 20	12 - 13	29 - 32.2	26 - 27	35 - 35	14 - 16
	MD L40	XX	17 - 28.1	26 - 30	11 - 11	13 - 14	17 - 20	17 - 18	15 - 17	12 - 13	30 - 32	27 - 29	36 - 37	14 - 16
	MD L41	XX	17 - 25.1	23 - 26	10 - 11	14 - 14	19 - 20	17 - 17	18 - 20	12 - 12	27.2 - 32.2	29 - 30	35 - 39	13 - 16
	MD L42	XX	25.1 - 28.1	24 - 25	12 - 12	14 - 16	16 - 19	7 - 17	18 - 19	12 - 13	30.2 - 31.2	29 - 42.2	37 - 37	14 - 15
	MD L43	XX	25.1 - 28.1	21 - 24	11 - 11	12 - 14	20 - 20	8 - 15	19 - 19	12 - 13	29 - 31.2	29 - 42.2	37 - 43.3	14 - 14
	MD L44	XX	18 - 22.1	21 - 21	10 - 11	12 - 14	18 - 21	16 - 16	18 - 20	13 - 14	27 - 30.2	27 - 39.2	? - ?	14 - 15
	MD L48	XX	26.1 - 26.1	20 - 33	11 - 11	13 - 16	17 - 19	7 - 13	17 - 21	12 - 12	29.2 - 29.2	26 - 44.2	36 - 38.3	13 - 14
	MD L51	XX	28.1 - 28.1	20.1 - 23	10 - 10	12 - 15	19 - 20	8 - 18	17 - 19	11 - 13	29.2 - 32	28 - 43.2	36 - 36	14 - 16
	MD L53	XX	18 - 26.1	27 - 28	10 - 12	12 - 16	18 - 20	8 - 18	19 - 20	12 - 13	28.2 - 32	26 - 38.2	33 - 40.3	14 - 17
	MD L59	XX	17 - 26.1	22 - 24	10 - 10	12 - 14	19 - 20	8 - 18	18 - 20	13 - 13	29.2 - 30.2	28 - 44.2	34 - 39.3	14 - 14
	MD S04	XX	25.1 - 25.1	18 - 19	11 - 12	13 - 16	16 - 17	8 - 16	18 - 20	12 - 13	29.2 - 30.2	29 - 44.2	36 - 36	16 - 17
	MD S11	XX	23.1 - 25.1	25 - 31	11 - 12	13 - 14	18 - 19	8 - 8	15 - 19	13 - 14	27.2 - 33	28 - 28	36 - 36	14 - 16
	MD S12	XX	18 - 28.1	28 - 31	10 - 12	12 - 14	18 - 19	7 - 17	18 - 19	13 - 13	28 - 34	26 - 40.2	32 - 40.3	14 - 16
	MD S14	XX	29.1 - 25.1	19 - 21	11 - 12	14 - 15	18 - 20	8 - 8	20 - 20	12 - 14	29.2 - 31.2	29 - 41.2	39.3 - 39.3	15 - 16
	MD S15	XX	24.1 - 24.1	20 - 33	12 - 12	13 - 14	17 - 19	8 - 17	18 - 19	11 - 12	29.2 - 30.2	29 - 44.2	39.3 - 40.3	14 - 14
	MD S17	XX	24 - 25.1	17 - 21.1	10 - 12	13 - 15	17 - 19	17 - 18	19 - 19	11 - 12	29.2 - 32.2	29 - 39.2	35 - 35	13 - 15
	MD S20	XX	26.1 - 27.1	22 - 22	10 - 12	13 - 14	18 - 18	8 - 18	19 - 19	13 - 15	26.2 - 30.2	34.2 - 43.2	33 - 36	14 - 14
	MD S21	XX	24 - 28.1	24 - 30	10 - 11	13 - 14	17 - 18	8 - 17	17 - 20	13 - 15	30 - 30	25 - 28	35 - 37	15 - 15
	MD S26	XX	18 - 18	23 - 23	10 - 12	14 - 15	19 - 19	17 - 18	18 - 18	13 - 13	29 - 33	26 - 27	36 - 36	15 - 16
	MD S28	XX	27.1 - 27.1	19 - 24	11 - 12	13 - 14	20 - 20	17 - 17	19 - 21	14 - 14	28.2 - 32	29 - 44.2	37.3 - 37.3	14 - 15
	MD X8	XX	19 - 26.1	28 - 32	10 - 11	12 - 13	17 - 17	8 - 16	17 - 19	13 - 14	31 - 32.2	30 - 31	35 - 36	15 - 16
Zamora														
Aliste	Z19	XY	24.1	24	10	13	20	17	21	10	27.2	28	38	15
	Z22	XY	27.1	27	12	14	18	19	18	14	30	26	45.3	15
	Z87	XY	24.1	20	12	14	18	16	19	12	28.2	29	36	14
	Z99	XY	25.1	30	10	13	18	8	19	11	29.2	28	37	15

Z154	XY	26.1	24	11	16	20	20	19	13	27.2	28	37	14
Z156	XY	25.1	23	12	14	19	18	19	12	28.2	26	38	15
Z164	XY	25.1	25.1	10	15	19	16	19	12	29	26	37	15
Z213	XY	25.1	17	12	14	21	17	18	12	27.2	32	30	14
Z231	XY	13.3	22	10	13	21	16	20	11	29.2	29	35	13
Z241	XY	25.1	22	12	14	18	19	19	12	30.2	44.2	35	15
Z246	XY	24.1	21	10	15	20	8	18	12	29.2	29	34	15
Z272	XY	19	20	12	13	20	16	19	13	28	25	38	15
Z287	XY	24	27	12	13	21	19	19	13	28.2	28	36	13
Z304	XY	30.1	20	12	14	19	7	18	11	32	29	34	13
Z307	XY	23	27	11	14	19	18	19	14	30.2	24	35	14
Z308	XY	24.1	23.1	11	15	19	8	19	13	31.2	30	36	14
Z309	XY	18	19.1	10	12	22	17	18	12	31.2	30	39.3	15
Z310	XY	18	21.1	10	15	20	17	18	14	35	40.2	37	15
Z311	XY	26.1	25	10	14	16	18	18	13	32	29	36	16
Z315	XY	23.1	25	11	13	21	18	19	13	31.2	27	34	15
Z318	XY	23	21.1	11	14	20	18	20	12	30.2	41.2	37	15
Z319	XY	23	20	10	13	21	17	19	12	30.2	40.2	35	16
Z320	XY	28.1	22	11	16	17	18	17	13	30	29	37	15
Z321	XY	25.1	29	12	15	15	16	19	12	28	27	38	15
Z322	XY	26.1	30	12	15	19	18	19	12	30.2	30	38	15
Z29	XX	23.1 - 23.1	22 - 25	10 - 11	12 - 13	20 - 22	14 - 16	16 - 19	12 - 14	27 - 32	24 - 27	35 - 37	13 - 15
Z84	XX	26.1 - 27.1	21 - 22	12 - 12	13 - 14	16 - 20	17 - 18	17 - 19	13 - 14	30 - 32.2	28 - 36.2	34 - 37	15 - 15
Z120	XX	24.1 - 27.1	21 - 23.1	10 - 11	13 - 15	20 - 21	17 - 17	16 - 18	12 - 13	28.2 - 31	26 - 26	35 - 36	14 - 15
Z173	XX	23 - 24.1	22 - 24	10 - 12	12 - 14	19 - 20	8 - 17	18 - 19	12 - 13	31.2 - 32	29 - 30	35 - 35	15 - 15
Z174	XX	25.1 - 28.1	17 - 31	10 - 12	13 - 17	18 - 21	8 - 19	18 - 19	11 - 13	29.2 - 30.2	27 - 27	37 - 38	13 - 14
Z218	XX	22.1 - 25.1	21.1 - 26	10 - 12	13 - 13	20 - 20	15 - 17	19 - 19	12 - 12	28 - 28.2	29 - 40.2	33 - 42.3	14 - 15
Z223	XX	18 - 26.1	22 - 28	10 - 11	14 - 16	19 - 19	15 - 16	19 - 19	13 - 13	28 - 30.2	26 - 30	38 - 40.3	13 - 15
Z228	XX	24.1 - 26.1	26 - 28	11 - 12	13 - 14	18 - 19	16 - 18	17 - 19	13 - 13	28 - 30	25 - 41.2	36 - 37.2	13 - 14
Z234	XX	18 - 24.1	22 - 29	10 - 11	15 - 16	21 - 21	16 - 19	17 - 20	13 - 13	30.2 - 31	25 - 27	36 - 37	14 - 15
Z236	XX	23 - 24.1	19.1 - 20	10 - 11	14 - 15	19 - 20	8 - 8	19 - 19	11 - 12	28 - 29.2	28 - 41.2	38 - 43.3	14 - 16

Bajo-Duero	Z237	XX	26.1 - 29.1	20 - 28	10 - 11	14 - 16	19 - 21	16 - 18	18 - 18	11 - 12	27.2 - 27.2	40.2 - 45.2	33.1 - 40.3	16 - 16
	Z244	XX	23.1 - 25.1	23 - 27	11 - 12	13 - 14	20 - 21	16 - 18	16 - 16	12 - 13	29.2 - 30	26 - 28	35 - 36	14 - 14
	Z245	XX	19 - 27.1	21 - 22	12 - 12	14 - 15	20 - 20	8 - 18	17 - 19	12 - 13	29 - 32	28 - 40.2	37 - 38	15 - 16
	Z8	XY	18	24	11	13	21	17	18	12	29	46.2	36	15
	Z34	XY	19	22	12	11	19	16	19	15	31.2	29	38.3	15
	Z67	XY	25.1	24	11	14	21	8	19	11	27	47.2	38.3	13
	Z115	XY	18	21	11	13	19	17	18	11	27.2	41.2	36	14
	Z131	XY	27.1	22.1	11	14	19	16	19	11	29.2	45.2	37	15
	Z151	XY	18	20.1	10	15	21	8	19	12	29	29	36	15
	Z159	XY	18	22.1	11	13	18	8	15	13	30	30	35	15
	Z206	XY	25.1	26	10	12	21	19	20	12	32.2	27	35	14
	Z222	XY	25.1	19.1	11	14	16	16	18	12	27.2	28	37.3	16
	Z357	XY	24.1	18	10	13	20	19	18	12	27.2	26	36	16
	Z362	XY	18	18	12	14	19	8	19	12	30.2	27	33	15
	Z364	XY	22.1	23	10	14	22	19	19	12	27.2	27	33	14
	Z2	XX	25.1 - 29.1	24 - 30	12 - 12	13 - 14	15 - 18	18 - 19	17 - 18	12 - 12	30 - 31	29 - 40.2	35 - 39.3	15 - 16
	Z18	XX	27.1 - 27.1	17.1 - 24.1	11 - 12	13 - 14	17 - 20	17 - 18	19 - 20	11 - 13	29.2 - 30.2	25 - 39.2	36 - 37	16 - 16
Benavente	Z21	XX	18 - 23	24 - 26	10 - 11	14 - 15	20 - 20	15 - 18	18 - 19	12 - 14	28.2 - 32	27 - 32	30 - 37	14 - 14
	Z31	XX	25.1 - 26.1	19 - 24	11 - 12	13 - 15	20 - 22	15 - 15	19 - 20	12 - 13	27.2 - 28.2	26 - 28	35 - 36	14 - 15
	Z37	XX	22.1 - 27.1	21 - 26	10 - 12	12 - 14	19 - 21	16 - 17	19 - 19	11 - 12	28 - 29.2	26 - 29	35 - 38.2	13 - 16
	Z53	XX	22 - 25.1	21 - 22	10 - 10	13 - 14	18 - 21	17 - 17	19 - 19	13 - 13	29.2 - 32	29 - 30	36 - 41.3	15 - 15
	Z66	XX	27.1 - 27.1	18 - 27	10 - 12	13 - 14	16 - 19	15 - 16	17 - 18	13 - 13	25 - 30.2	30 - 40.2	35 - 40.3	13 - 16
	Z90	XX	24.1 - 24.1	22.1 - 23.1	10 - 12	12 - 14	19 - 21	17 - 18	18 - 19	12 - 12	27.2 - 30.2	31 - 43.2	34 - 36	15 - 15
	Z96	XX	23 - 23	18 - 23.1	10 - 10	13 - 14	18 - 19	9 - 18	17 - 19	12 - 14	28.2 - 31	27 - 30	34 - 35	13 - 16
	Z108	XX	24.1 - 24.1	20 - 24	9 - 11	14 - 15	19 - 22	17 - 18	16 - 19	12 - 12	29.2 - 32.2	25 - 40.2	36 - 39.3	15 - 15
	Z126	XX	24 - 26.1	24 - 28	11 - 12	14 - 14	18 - 19	15 - 17	19 - 19	11 - 12	28.2 - 30.2	28 - 38.2	39.3 - 43.3	15 - 17
	Z178	XX	22.1 - 24.1	24 - 31	10 - 11	15 - 16	17 - 20	8 - 15	18 - 21	10 - 13	28.2 - 30.2	28 - 28	34 - 38	14 - 14
	Z224	XX	18 - 22.1	24 - 29	11 - 12	13 - 14	19 - 21	17 - 17	19 - 19	12 - 15	30.2 - 30.2	22 - 27	35 - 37	15 - 15
	Z260	XX	23.1 - 25.1	22 - 24	11 - 11	14 - 16	20 - 22	16 - 19	17 - 20	12 - 14	29.2 - 31	26 - 29	35 - 38	14 - 14
	Z7	XY	25.1	21	10	15	20	18	16	15	31	28	34	16
	Z70	XY	26.1	17.1	10	14	20	17	18	13	32	30	37	16

Z111	XY	24.1	25	11	15	22	18	16	13	31	31	42.3	14
Z132	XY	26.1	20	11	14	18	18	21	13	30.2	29	38	13
Z210	XY	32.1	24	10	14	21	21	19	13	30.2	39.2	38	13
Z230	XY	28.1	17	11	14	21	17	20	12	28.2	28	34	14
Z281	XY	26.1	22	13	13	21	18	17	15	31	25	36	15
Z327	XY	26.1	22.1	10	15	20	16	19	13	31.2	28	37	14
Z328	XY	23.1	28	11	15	21	17	17	14	30	39.2	42.3	15
Z332	XY	26.1	23	10	14	18	16	19	13	31.2	30	40.3	15
Z333	XY	26.1	24	12	15	18	15	20	14	27.2	28	37	15
Z334	XY	18	32.1	10	14	20	15	19	13	27.2	26	36	13
Z337	XY	30.1	23.1	11	13	21	15	19	12	30.2	40.2	35	14
Z338	XY	24.1	25	12	14	21	16	18	14	30	28	37	15
Z339	XY	18	18.1	11	13	18.2	8	18	12	27.2	28	36	15
Z340	XY	26.1	24	10	13	20	17	17	12	26.2	29	37	15
Z341	XY	22.1	20	10	12	19	18	19	14	30.2	42.2	41.3	14
Z344	XY	23.1	28	11	12	21	18	20	12	28.2	30	36	16
Z356	XY	23.1	29	11	14	23	15	18	14	32	27	35	12
Z383	XY	25.1	24	11	14	19	19	19	12	29	27	35	13
Z3	XX	24.1 - 25.1	17 - 22.1	11 - 12	11 - 12	19 - 19	8 - 17	17 - 17	14 - 15	31 - 33	24 - 40.2	35 - 37	14 - 14
Z11	XX	23 - 25.1	? - 25	10 - 12	13 - 13	15 - 20	17 - 17	17 - 18	14 - 14	31 - 32	28 - 28	35 - 36	15 - 16
Z16	XX	22.1 - 23.1	24 - 25	10 - 11	14 - 14	19 - 23	16 - 19	17 - 17	14 - 14	31 - 31	27 - 40.2	37.3 - 39	15 - 16
Z137	XX	24.1 - 26.1	23 - 24	12 - 13	14 - 14	19 - 19	16 - 17	16 - 17	11 - 15	30 - 31	28 - 40.2	34 - 40.3	15 - 16
Z141	XX	18 - 24	20 - 23	12 - 12	14 - 15	18 - 19	7 - 8	18 - 19	11 - 14	29.2 - 29.2	25 - 27	34 - 36	14 - 15
Z179	XX	23 - 26.1	17 - 22	10 - 10	11 - 14	20 - 20	16 - 17	17 - 17	12 - 13	26.2 - 31	31 - 36.2	31 - 33	15 - 15
Z182	XX	25.1 - 26.1	25 - 28	10 - 12	13 - 14	16 - 20	7 - 18	18 - 19	11 - 13	30.2 - 30.2	28 - 43.2	35 - 36	14 - 16
Z201	XX	19 - 27.1	24 - 24	11 - 12	14 - 15	19 - 20	7 - 8	18 - 19	12 - 14	30 - 31.2	28 - 43.2	34 - 37	14 - 14
Z217	XX	19 - 26.1	24 - 27	9 - 12	12 - 14	18 - 21	8 - 15	18 - 20	11 - 12	29.2 - 31.2	29 - 41.2	35 - 37	14 - 15
Z227	XX	221 - 26.1	22 - 31	12 - 12	14 - 14	19 - 20	8 - 16	16 - 20	11 - 12	28.2 - 31.2	28 - 41.2	34.2 - 40.3	14 - 15
Z229	XX	23 - 26.1	21 - 31	10 - 11	13 - 13	18 - 20	7 - 18	17 - 18	12 - 14	24.2 - 29	27 - 41.2	36 - 38	14 - 14
Z251	XX	24 - 24.1	23 - 28	10 - 10	14 - 14	18 - 22	16 - 17	15 - 19	12 - 12	31.2 - 32	27 - 31	37 - 39.3	14 - 15
Z263	XX	18 - 22.1	22.1 - 23	10 - 12	13 - 14	18 - 21	8 - 16	18 - 19	11 - 13	30 - 31	40.2 - 40.2	40.3 - 40.3	13 - 13

Campos-Pan	Z265	XX	23 - 25.1	18 - 20	10 - 10	12 - 15	17 - 19	15 - 16	19 - 20	13 - 13	31.2 - 33	26 - 30	35 - 36	14 - 16
	Z268	XX	18 - 27.1	20 - 20	10 - 12	12 - 14	18 - 20	8 - 16	16 - 16	11 - 11	30 - 30	30 - 30	36 - 36	14 - 15
	Z273	XX	18 - 21	20 - 21	10 - 10	14 - 15	19 - 21	14 - 19	19 - 19	12 - 12	27.2 - 28	31 - 41.2	35 - 40.3	14 - 15
	Z38	XY	26.1	21	9	12	18	9	18	12	30.2	29	35	15
	Z41	XY	26.1	27	11	13	22	18	19	12	32.2	27	35	14
	Z44	XY	18	20	12	13	20	18	19	11	31.2	38.2	41.3	15
	Z46	XY	19	28	10	13	22	16	15	13	34	40.2	40.3	15
	Z49	XY	27.1	32	11	15	21	16	18	12	30.2	38.2	36	14
	Z54	XY	18	22	11	12	16	8	17	13	31	31	35	15
	Z75	XY	22	25	10	14	20	17	19	14	31.2	27	37.3	16
	Z79	XY	26.1	18.1	12	16	19	18	19	13	33	30	36	14
	Z106	XY	18	28	12	14	20	16	16	12	28.2	29	36	15
	Z114	XY	26.1	29	12	13	20	15	17	12	33	31	42.3	14
	Z127	XY	27.1	19	13	13	21	17	17	14	33	28	35	15
	Z130	XY	23	22	10	14	20	16	17	16	31	41.2	37.2	14
	Z160	XY	22	21.1	11	14	21	18	16	13	33	29	41.3	14
	Z176	XY	26.1	20	10	14	19	15	19	12	28	44.2	38.3	14
	Z177	XY	28.1	22	10	13	20	17	17	12	32	39.2	38	15
	Z198	XY	26.1	22	11	14	18	17	18	12	28	29	40.3	14
	Z249	XY	23	21	11	12	19	16	19	11	29.2	35.2	37	14
	Z264	XY	27.1	20	11	12	22	15	19	11	28.2	29	41.3	14
	Z342	XY	26.1	25	11	12	19	17	19	13	30.2	46.2	34	14
	Z373	XY	24.1	21	10	13	18	17	19	13	31	36.2	31	15
	Z378	XY	28.1	27	11	13	20	16	19	12	27.2	28	36	15
	Z379	XY	25.1	22	13	14	19	8	19	12	28	27	36	15
	Z380	XY	26.1	27	12	16	19	19	19	13	30.2	41.2	35	15
	Z381	XY	18	24	10	15	16	16	16	13	28.2	29	36	15
	Z382	XY	19	21	10	15	20	18	20	12	28	26	35	16
	Z384	XY	27.1	19.1	10	13	20	18	17	16	31	41.2	35	16
	Z391	XY	24.1	32	10	15	21	8	19	13	28	30	36	17
	Z399	XY	26.1	22	11	13	20	18	20	15	33	42.2	34	14

Sanabria	Z9	XX	18 - 24.1	24 - 30	10 - 11	12 - 12	19 - 19	15 - 17	19 - 19	12 - 12	27.2 - 30.2	29 - 29	33 - 35	14 - 15
	Z10	XX	18 - 26.1	20 - 25	12 - 12	15 - 15	18 - 20	7 - 17	18 - 18	11 - 11	29.2 - 29.2	26 - 28	31 - 37	15 - 15
	Z30	XX	25.1 - 25.1	25 - 27	11 - 12	13 - 15	18 - 20	8 - 9	20 - 21	13 - 13	27.2 - 31.2	25 - 26	35 - 39.3	15 - 15
	Z56	XX	24.1 - 26.1	29 - 30	11 - 12	14 - 14	19 - 19	16 - 16	18 - 19	12 - 14	28.2 - 32	24 - 29	36 - 36	13 - 15
	Z101	XX	18 - 26.1	24 - 26	10 - 11	11 - 13	19 - 20	9 - 16	16 - 18	11 - 14	31 - 31.2	31 - 31	34 - 36	15 - 16
	Z110	XX	24.1 - 27.1	28 - 32	10 - 11	13 - 13	17 - 20	9 - 16	19 - 19	12 - 12	30.2 - 30.2	29 - 29	33 - 34	15 - 15
	Z125	XX	18 - 26.1	24 - 28	10 - 11	13 - 14	18 - 20	8 - 16	19 - 19	12 - 12	28 - 30.2	29 - 452	36 - 39	14 - 15
	Z133	XX	23.1 - 25.1	20 - 21	10 - 12	14 - 15	18 - 19	8 - 15	18 - 20	12 - 14	30.2 - 32	26 - 30	37 - 41.3	15 - 15
	Z134	XX	23.1 - 24.1	20 - 21.1	10 - 11	13 - 15	18 - 20	8 - 15	19 - 19	11 - 13	28.2 - 29.2	27 - 40.2	36 - 36.2	14 - 15
	Z138	XX	23 - 24.1	20 - 20.1	10 - 12	14 - 14	19 - 19	17 - 17	18 - 20	11 - 14	27.2 - 31.2	26 - 28	36 - 37	15 - 15
	Z142	XX	23.1 - 25.1	20 - 20	11 - 11	13 - 14	20 - 20	16 - 17	19 - 19	11 - 13	28.2 - 29.2	27 - 42.2	36.2 - 39.3	15 - 16
	Z145	XX	20 - 22	18 - 22.1	10 - 10	13 - 13	18 - 20	16 - 17	17 - 18	12 - 16	30 - 30.2	29 - 30	36 - 37	14 - 15
	Z146	XX	26.1 - 29.1	19 - 23	10 - 11	15 - 15	17 - 17	8 - 17	18 - 19	12 - 14	26.2 - 33	28 - 29	35 - 37	15 - 15
	Z163	XX	26.1 - 27.1	22 - 33	10 - 11	14 - 16	18 - 19	8 - 17	19 - 19	12 - 12	31.2 - 32.2	29 - 38.2	36 - 41.3	14 - 14
	Z165	XX	24.1 - 27.1	18 - 27	10 - 12	14 - 15	20 - 21	15 - 16	18 - 21	11 - 13	30.2 - 32	25 - 39.2	35 - 40.3	13 - 14
	Z175	XX	19 - 27.1	22 - 22.1	11 - 11	12 - 14	17 - 22	13 - 16	16 - 19	13 - 16	26.2 - 31	25 - 26	35 - 36	13 - 15
	Z180	XX	24.1 - 26.1	21 - 25	10 - 12	12 - 13	18 - 19	16 - 19	18 - 19	13 - 13	29.2 - 31	29 - 30	34 - 38	15 - 16
	Z185	XX	24.1 - 24.1	21 - 28	10 - 10	13 - 14	18 - 20	18 - 19	18 - 19	11 - 13	28 - 29.2	26 - 44.2	36 - 39.3	14 - 15
	Z203	XX	20 - 24.1	23 - 26	10 - 11	13 - 13	18 - 19	17 - 19	19 - 20	11 - 13	29.2 - 30.2	27 - 29	37 - 38	14 - 15
	Z205	XX	18 - 27.1	22 - 23	10 - 11	12 - 13	18 - 20	16 - 18	16 - 17	12 - 14	28.2 - 33	27 - 43.2	35 - 38	15 - 16
	Z69	XY	18	22.1	11	14	17	8	19	12	31	29	35	13
	Z168	XY	26.1	26	11	14	21	17	18	13	30	39.2	37	15
	Z186	XY	18	31	12	14	19	15	20	16	31	27	37	14
	Z239	XY	27.1	24	11	14	19	16	17	14	30	28	35	15
	Z242	XY	24.1	22.1	10	11	20	16	21	13	33.2	29	34	16
	Z271	XY	23	22	10	15	19	18	17	15	33	27	35	15
	Z274	XY	18	30	10	14	16	16	19	12	28	29	35	15
	Z275	XY	28.1	26	11	14	20	15	19	12	29.2	28	37	15
	Z286	XY	24.1	23	12	15	18	19	18	12	27.2	42.2	35	16
	Z377	XY	25.1	25	10	13	17	17	19	11	29.2	29	37	14
	Z72	XX	17 - 24.1	22 - 27	12 - 12	13 - 14	21 - 22	15 - 16	16 - 19	13 - 14	31 - 33	25 - 39.2	35 - 38	15 - 15

Sayago	Z181	XX	18 - 26.1	22 - 28	11 - 12	13 - 15	17 - 18	19 - 19	18 - 18	14 - 14	31.2 - 32.2	29 - 30	35 - 36	15 - 15
	Z238	XX	25.1 - 27.1	24 - 29	11 - 11	14 - 14	16 - 18	17 - 19	19 - 19	12 - 12	28 - 29	29 - 31	36 - 39	15 - 16
	Z270	XX	24.1 - 24.1	23 - 27	10 - 12	13 - 14	19 - 20	8 - 16	17 - 17	13 - 14	29.2 - 31	28 - 28	35 - 38	14 - 14
	Z278	XX	26.1 - 27.1	24 - 28	10 - 11	15 - 15	19 - 19	8 - 17	17 - 19	14 - 15	29 - 33	28 - 40.2	37 - 40.3	13 - 15
	Z279	XX	25.1 - 26.1	18 - 23	10 - 11	12 - 12	17 - 18	14 - 17	16 - 17	13 - 14	31 - 33	44.2 - 44.2	36 - 37	15 - 15
	Z282	XX	23 - 27.1	21 - 24	11 - 11	12 - 14	18 - 19	8 - 9	17 - 19	12 - 12	28.2 - 31	26 - 44.2	35 - 38.3	14 - 14
	Z283	XX	24.1 - 25.1	28 - 30	11 - 12	14 - 14	17 - 19	8 - 20	17 - 18	13 - 14	31 - 33.2	40.2 - 45.2	34 - 38	14 - 16
	Z285	XX	24.1 - 24.1	23 - 23.1	10 - 12	14 - 15	20 - 21	15 - 18	18 - 19	11 - 13	30.2 - 31	25 - 27	36 - 39.3	14 - 15
	Z289	XX	18 - 28.1	26 - 31	11 - 12	14 - 15	15 - 19	16 - 19	17 - 19	13 - 13	30.2 - 32	26 - 39.2	35 - 36	15 - 16
	Z6	XY	25.1	26	12	13	17	19	19	12	28.2	29	36	15
	Z73	XY	26.1	23	10	13	16	17	17	14	31	44.2	37.3	15
	Z88	XY	18	29	10	13	18	17	16	12	33	24	39	14
	Z118	XY	26.1	20	12	14	19	17	19	12	28.2	28	35	15
	Z119	XY	24.1	22	12	12	20	8	19	14	31.2	43.2	37.3	14
	Z123	XY	18	22	10	15	18	8	20	12	29	29	36	15
	Z124	XY	27.1	25	11	13	19	15	20	13	32	28	35	15
	Z158	XY	23.1	23	10	13	15	18	19	11	30.2	32	34	13
	Z189	XY	27.1	28	12	12	18	17	20	13	30.2	29	36	15
	Z204	XY	19	25	11	14	19	17	19	11	31.2	25	36	17
	Z252	XY	25.1	31	12	13	19	17	19	12	30.2	30	34	15
	Z257	XY	27.1	24	11	14	19	17	20	11	27.2	28	35	15
	Z276	XY	25.1	26	10	13	22	16	18	13	33	29	36	15
	Z293	XY	28.1	18.1	12	14	19	17	19	12	29.2	29	37	15
	Z294	XY	24.1	26	11	13	18	17	17	15	30	28	37	15
	Z295	XY	27.1	23	13	15	22	17	19	12	28	31	32	15
	Z296	XY	23	21	11	13	20	17	17	15	30	28	35.3	15
	Z297	XY	27.1	17	10	14	20	18	19	12	27.2	41.2	36.2	14
	Z300	XY	26.1	27	11	14	20	17	18	12	32.2	30	36	15
	Z302	XY	24	23	12	14	16	17	19	11	28.2	39.2	39.3	15
	Z303	XY	27.1	20	11	14	19	16	19	12	30.2	29	38	16
	Z86	XX	25.1 - 25.1	20 - 29	11 - 11	14 - 16	19 - 21	18 - 18	18 - 20	12 - 14	31.2 - 31.2	26 - 29	36 - 38	13 - 15

Z98	XX	24.1 - 25.1	19 - 28	11 - 12	12 - 14	20 - 21	16 - 16	18 - 19	12 - 13	28.2 - 33	29 - 30	35 - 37	15 - 16
Z139	XX	18 - 24.1	19 - 20	10 - 11	13 - 14	18 - 21	16 - 17	19 - 19	14 - 14	31.2 - 32	27 - 29	36 - 37	13 - 15
Z144	XX	25.1 - 26.1	19 - 21.1	11 - 11	12 - 14	17 - 19	15 - 17	19 - 19	11 - 13	31.2 - 31.2	27 - 28	35 - 39	13 - 15
Z153	XX	24.1 - 25.1	19.1 - 30	10 - 12	14 - 15	18 - 19	8 - 16	18 - 19	12 - 13	30.2 - 31.2	27 - 30	34 - 35	15 - 15
Z166	XX	23 - 26.1	18 - 21	10 - 11	12 - 13	17 - 20	7 - 8	17 - 20	12 - 13	26.2 - 30	25 - 422	33 - 36	13 - 14
Z209	XX	18 - 27.1	23 - 27	11 - 12	11 - 15	19 - 19	16 - 16	19 - 19	11 - 12	29.2 - 29.2	27 - 28	35 - 35	15 - 15
Z220	XX	26.1 - 26.1	25 - 25	11 - 11	14 - 15	20 - 20	15 - 19	19 - 19	12 - 12	26.2 - 27.2	26 - 27	37 - 37	14 - 15
Z256	XX	23.1 - 24	17 - 22	10 - 12	12 - 15	20 - 20	16 - 19	19 - 20	12 - 13	27.2 - 32	28 - 32	34 - 37	15 - 15
Z288	XX	18 - 26.1	23 - 27	12 - 12	13 - 15	18 - 19	18 - 20	18 - 19	12 - 13	29 - 32.2	28 - 28	36 - 37	14 - 15

Table S2 Genetic profiles obtained with the amplification of the 32 X-Indels.

Sample		AM	MID 3736	MID 3730	MID 1361	MID 329	MID 3716	MID 3692	MID 2637	MID 3740	MID 198	MID 3703	MID 3690	MID 3722	MID 3732	MID 3712	MID 1736	MID 3719
Miranda do Douro	MD 02	XY	1	2	2	2	1	1	1	2	2	2	1	2	2	2	1	2
	MD 07	XY	1	2	2	2	1	2	1	1	1	1	1	1	2	1	1	2
	MD 09	XY	1	2	2	2	1	2	1	2	1	1	2	2	2	1	2	1
	MD 11	XY	1	2	2	2	1	2	1	1	2	2	2	2	1	2	1	2
	MD 19	XY	1	2	1	2	2	2	1	2	2	2	2	2	2	2	2	2
	MD 25	XY	1	2	2	1	1	2	1	1	1	2	1	2	1	2	2	2
	MD 50	XY	1	?	2	2	1	1	1	2	2	2	2	1	2	1	1	2
	MD 51	XY	1	2	1	2	1	1	1	1	1	2	2	2	2	2	2	2
	MD 58	XY	1	2	1	1	1	2	1	1	2	1	1	2	2	2	2	2
	MD 59	XY	1	2	2	2	1	2	2	1	1	2	2	2	1	2	1	2
	MD 61	XY	1	2	2	1	2	2	1	1	1	2	1	2	1	2	1	2
	MD 64	XY	1	2	2	2	1	2	1	2	1	2	2	2	2	2	1	2
	MD 73	XY	1	1	2	2	1	2	1	1	1	2	2	1	2	2	2	2
	MD 93	XY	1	2	1	2	1	2	1	2	2	1	2	1	2	2	2	2
	MD 94	XY	1	2	1	1	1	2	1	1	1	1	2	1	2	1	1	2
	MD L05	XY	1	2	2	1	1	2	2	2	1	2	2	2	2	2	1	2
	MD L09	XY	1	1	1	2	2	1	1	1	2	1	2	2	2	2	1	2
	MD L11	XY	1	1	2	2	2	2	1	2	2	1	2	1	2	2	2	2
	MD L12	XY	2	?	1	1	2	2	1	2	1	2	1	2	2	2	1	2
	MD L22	XY	1	1	2	1	2	2	1	2	2	1	1	2	2	2	2	2
	MD L25	XY	1	2	2	1	1	2	1	2	2	2	2	1	2	1	1	2
	MD L26	XY	2	2	2	2	1	2	1	1	2	2	1	2	2	2	1	2
	MD L29	XY	1	2	2	1	1	2	1	1	2	2	2	2	2	2	2	2
	MD L32	XY	1	1	2	2	2	2	1	2	1	2	2	2	1	1	1	2
	MD L34	XY	1	1	2	2	1	2	1	1	1	2	2	2	1	2	2	1
	MD L35	XY	1	2	2	2	2	2	1	2	1	2	2	2	2	2	2	2

	MD L38	XY	2	2	2	2	1	1	1	2	2	2	2	2	2	2	1	2
	MD L39	XY	1	2	2	2	1	2	2	1	2	2	1	2	2	2	2	2
	MD L46	XY	1	2	2	2	2	2	1	1	1	2	2	1	1	2	1	2
	MD L49	XY	1	?	2	2	2	2	2	2	2	2	1	1	2	2	1	2
	MD L56	XY	2	2	2	1	2	2	1	2	1	2	1	2	2	2	1	2
	MD L57	XY	1	2	2	2	1	1	1	2	1	2	2	1	2	2	1	1
	MD L60	XY	1	2	2	2	1	1	1	1	1	2	1	2	2	2	2	2
	MD S1	XY	2	2	2	2	1	1	1	1	1	2	1	2	1	1	1	2
	MD S2	XY	1	1	2	1	1	2	1	2	2	1	1	2	2	2	2	2
	MD S5	XY	1	1	1	2	1	1	1	2	2	1	2	2	2	2	2	2
	MD S7	XY	1	1	2	2	2	2	1	1	2	1	1	1	1	2	1	2
	MD S8	XY	2	2	2	2	2	2	1	2	2	2	2	2	2	1	2	2
	MD S9	XY	1	2	2	2	1	2	1	2	1	1	2	2	2	2	1	2
	MD S10	XY	1	2	2	2	1	2	1	1	2	2	1	2	2	2	1	2
	MD S13	XY	1	1	2	1	2	2	1	2	1	2	1	2	1	1	1	2
	MD S16	XY	1	1	2	1	1	1	1	1	1	2	2	1	1	2	2	2
	MD S24	XY	2	1	1	2	1	2	1	1	1	2	2	2	2	1	2	2
	MD S27	XY	2	2	2	1	1	1	1	2	2	2	1	1	2	2	2	2
	MD S30	XY	2	2	2	1	2	2	1	2	1	2	2	2	2	2	2	2
	MD S31	XY	2	2	2	1	2	2	1	1	1	2	1	2	1	2	1	2
	MD S32	XY	2	2	2	1	2	2	1	2	2	2	2	1	2	2	2	2
	MD S33	XY	?	2	2	2	1	2	1	2	1	2	2	2	2	1	1	2
	MD S34	XY	1	2	2	1	1	2	1	2	1	2	2	2	2	2	1	2
	MD S35	XY	2	2	2	2	1	2	1	1	2	2	1	1	2	2	1	2
	MD S36	XY	1	2	2	2	2	2	1	2	1	2	2	2	2	2	1	2
	MD X3	XY	?	2	2	1	1	2	1	2	1	2	2	1	2	2	1	2
	MD X6	XY	2	1	2	1	1	1	2	2	1	1	1	2	2	1	2	2
	MD X7	XY	1	1	2	2	1	2	1	1	1	2	2	1	1	2	2	2
	MD 4	XY	1	2	2	2	1	1	1	2	2	2	1	2	2	2	1	2
	MD 5	XX	1 - 2	1 - 1	2 - 2	1 - 1	1 - 1	2 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 2	2 - 2	1-2	2-2	1-2	2-2

	MD 6	XX	1 - 1	1 - 2	2 - 2	1 - 2	1 - 2	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2	1 - 2	2 - 2	2 - 2	2 - 2	1 - 2	1 - 2
	MD 8	XX	1 - 2	1 - 2	2 - 2	2 - 2	1 - 2	2 - 2	1 - 1	1 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 1	2 - 2	1 - 2	2 - 2
	MD 10	XX	2 - 2	1 - 2	2 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 2	2 - 2	1 - 2	2 - 2	1 - 1	1 - 2	1 - 1	1 - 2
	MD 12	XX	1 - 2	2 - 2	2 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 2	2 - 2	1 - 2	1 - 1	1 - 1	1 - 2
	MD 15	XX	1 - 1	1 - 1	2 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 1	2 - 2	1 - 2	2 - 2	1 - 2	2 - 2	2 - 2	2 - 2
	MD 17	XX	1 - 2	1 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 2	2 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2
	MD 18	XX	1 - 1	1 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2	1 - 1	1 - 2	1 - 2	2 - 2	2 - 2	2 - 2	1 - 2	2 - 2
	MD 20	XX	1 - 1	1 - 2	2 - 2	1 - 1	1 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 2	2 - 2	2 - 2	1 - 2	2 - 2	1 - 2
	MD 21	XX	2 - 2	1 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 2	1 - 2	2 - 2	2 - 2	2 - 2	2 - 2	1 - 2	2 - 2
	MD 26	XX	1 - 2	2 - 2	2 - 2	1 - 2	1 - 1	1 - 1	1 - 1	1 - 1	1 - 1	2 - 2	2 - 2	2 - 2	1 - 1	2 - 2	2 - 2	2 - 2
	MD 27	XX	1 - 2	1 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 1	1 - 1	1 - 2	2 - 2	1 - 2	2 - 2	2 - 2	1 - 2
	MD 48	XX	1 - 1	2 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1 - 1	2 - 2	1 - 2	1 - 2	1 - 2	2 - 2
	MD 54	XX	1 - 1	2 - 2	1 - 2	1 - 2	2 - 2	2 - 2	1 - 1	1 - 2	1 - 2	2 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 2	2 - 2
	MD 55	XX	1 - 2	2 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1 - 1	1 - 2	1 - 2	2 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 1	2 - 2
	MD 56	XX	1 - 1	2 - 2	2 - 2	2 - 2	1 - 2	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2	1 - 2	2 - 2	1 - 2	1 - 1	1 - 2	1 - 2
	MD 57	XX	1 - 1	2 - 2	2 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 1	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2	2 - 2	1 - 2	2 - 2
	MD 60	XX	1 - 2	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2	1 - 2	1 - 1	1 - 2	1 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 2	2 - 2
	MD 62	XX	2 - 2	1 - 2	2 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 2	1 - 1	1 - 1	1 - 2	1 - 2	2 - 2	2 - 2	2 - 2	2 - 2
	MD 63	XX	1 - 1	1 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 1	2 - 2	2 - 2	2 - 2	2 - 2	1 - 1	1 - 2	2 - 2	2 - 2	2 - 2
	MD 65	XX	1 - 1	1 - 2	1 - 2	2 - 2	1 - 1	1 - 2	1 - 1	1 - 1	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2	2 - 2	1 - 2	2 - 2
	MD 66	XX	1 - 2	2 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 1	1 - 1	1 - 1	1 - 2	2 - 2	2 - 2	2 - 2	2 - 2	1 - 2	1 - 2
	MD 67	XX	1 - 2	2 - 2	1 - 2	2 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 1	2 - 2	2 - 2	1 - 2
	MD 69	XX	1 - 2	1 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	1 - 2	1 - 2	2 - 2	2 - 2	1 - 2	2 - 2	2 - 2	1 - 2	1 - 2
	MD 70	XX	1 - 1	2 - 2	1 - 2	1 - 2	1 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 2	2 - 2
	MD 71	XX	1 - 2	2 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 1	1 - 1	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	2 - 2
	MD 72	XX	1 - 1	2 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 2	1 - 2	2 - 2	2 - 2	1 - 2	2 - 2	1 - 2	2 - 2
	MD 74	XX	1 - 2	1 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 2	2 - 2
	MD 89	XX	1 - 1	2 - 2	2 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 1	1 - 2	2 - 2	1 - 2	2 - 2	1 - 2	2 - 2	1 - 2	2 - 2
	MD 90	XX	1 - 2	1 - 2	1 - 2	2 - 2	1 - 1	1 - 2	1 - 1	1 - 1	1 - 2	1 - 1	1 - 1	1 - 1	1 - 2	2 - 2	1 - 2	2 - 2
	MD L01	XX	1 - 2	1 - 2	2 - 2	1 - 2	1 - 1	2 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2

	MD L03	XX	1 - 2	2 - 2	2 - 2	1 - 1	1 - 2	2 - 2	1 - 2	2 - 2	1 - 1	2 - 2	2 - 2	2 - 2	1-2	1-1	1-1	1-2
	MD L06	XX	1 - 1	1 - 2	2 - 2	2 - 2	2 - 2	1 - 2	1 - 1	1 - 1	1 - 2	1 - 2	2 - 2	2 - 2	2-2	2-2	1-1	2-2
	MD L08	XX	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2	1 - 2	1 - 1	1 - 1	1 - 2	1 - 2	2 - 2	2-2	2-2	1-2	1-2
	MD L10	XX	1 - 2	1 - 2	2 - 2	1 - 1	1 - 2	2 - 2	1 - 2	1 - 2	1 - 2	2 - 2	2 - 2	1 - 2	2-2	2-2	2-2	1-2
	MD L13	XX	2 - 2	1 - 2	1 - 2	2 - 2	2 - 2	2 - 2	1 - 1	1 - 1	1 - 2	2 - 2	2 - 2	2 - 2	2-2	1-1	1-2	1-2
	MD L14	XX	1 - 2	1 - 1	2 - 2	2 - 2	2 - 2	2 - 2	1 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 1	1-2	1-2	2-2	2-2
	MD L15	XX	1 - 2	1 - 2	2 - 2	1 - 2	1 - 1	1 - 2	1 - 1	2 - 2	1 - 2	1 - 1	2 - 2	2 - 2	2-2	2-2	2-2	2-2
	MD L16	XX	1 - 2	1 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 2	2 - 2	1-2	1-2	2-2	2-2
	MD L17	XX	1 - 1	1 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2	1 - 1	2 - 2	2 - 2	2 - 2	1-1	1-2	1-2	1-2
	MD L20	XX	1 - 1	2 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 1	1 - 2	1 - 1	1 - 2	2 - 2	2 - 2	2-2	1-2	1-1	2-2
	MD L23	XX	1 - 2	1 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 1	1 - 2	2-2	2-2	1-1	2-2
	MD L28	XX	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2	1 - 1	1 - 2	1 - 2	2 - 2	1 - 2	1 - 2	1-2	2-2	1-1	2-2
	MD L36	XX	1 - 1	2 - 2	2 - 2	1 - 2	1 - 2	1 - 2	1 - 1	1 - 2	1 - 2	2 - 2	2 - 2	1 - 1	1-2	1-2	1-1	1-2
	MD L37	XX	1 - 1	2 - 2	1 - 2	1 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 2	2 - 2	2 - 2	2 - 2	1-2	2-2	2-2	2-2
	MD L40	XX	1 - 1	1 - 2	1 - 2	2 - 2	1 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 2	1 - 2	2-2	2-2	1-2	1-2
	MD L41	XX	1 - 2	1 - 2	1 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 2	1 - 2	2-2	2-2	1-2	1-2
	MD L42	XX	1 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 1	1 - 1	1 - 1	2 - 2	1 - 1	1 - 2	2-2	2-2	2-2	2-2
	MD L43	XX	1 - 2	1 - 2	2 - 2	2 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1 - 2	2 - 2	2 - 2	2 - 2	2-2	2-2	1-2	2-2
	MD L44	XX	1 - 2	? - ?	2 - 2	1 - 2	1 - 1	2 - 2	1 - 2	2 - 2	1 - 2	2 - 2	1 - 1	1 - 1	2-2	?-?	2-2	?-?
	MD L48	XX	1 - 1	1 - 2	2 - 2	1 - 2	1 - 1	1 - 2	1 - 1	2 - 2	1 - 2	2 - 2	2 - 2	1 - 1	2-2	2-2	2-2	1-2
	MD L51	XX	? - ?	2 - 2	1 - 2	2 - 2	2 - 2	1 - 2	1 - 1	1 - 2	2 - 2	1 - 2	1 - 2	1 - 2	1-2	1-2	1-1	2-2
	MD L53	XX	1 - 2	1 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 2	2 - 2	1 - 2	1 - 2	1-2	2-2	1-2	2-2
	MD L59	XX	1 - 1	2 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 2	2 - 2	2-2	1-2	1-2	1-2
	MD S4	XX	1 - 2	2 - 2	1 - 2	2 - 2	1 - 2	2 - 2	1 - 2	1 - 1	1 - 1	1 - 2	2 - 2	2 - 2	2-2	2-2	1-2	2-2
	MD S11	XX	1 - 1	2 - 2	1 - 2	2 - 2	1 - 2	2 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 2	2 - 2	1-2	1-2	1-2	2-2
	MD S12	XX	1 - 2	1 - 2	1 - 1	2 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 1	2 - 2	1 - 1	1 - 1	2-2	2-2	1-2	2-2
	MD S14	XX	1 - 1	1 - 1	2 - 2	2 - 2	1 - 2	2 - 2	1 - 2	1 - 2	1 - 1	2 - 2	2 - 2	2 - 2	1-1	1-2	1-2	2-2
	MD S15	XX	1 - 2	2 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1 - 1	2 - 2	1-2	1-2	1-2	2-2
	MD S17	XX	1 - 2	2 - 2	1 - 2	1 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1-2	2-2	1-2	2-2
	MD S20	XX	1 - 1	2 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 1	1 - 1	1 - 2	1 - 1	2 - 2	2 - 2	1-1	2-2	1-2	2-2

	MD S21	XX	2 - 2	2 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 2	2 - 2	1-1	2-2	2-2	2-2
	MD S26	XX	1 - 1	1 - 2	1 - 1	1 - 2	1 - 1	2 - 2	1 - 1	2 - 2	1 - 2	1 - 2	1 - 2	1 - 1	2-2	2-2	2-2	1-2
	MD S28	XX	1 - 1	1 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 2	2 - 2	1 - 2	1 - 2	1 - 2	2 - 2	1-2	2-2	2-2	2-2
	MD X8	XX	2 - 2	1 - 2	2 - 2	2 - 2	1 - 2	1 - 2	1 - 2	1 - 2	1 - 1	1 - 2	1 - 1	2 - 2	1-2	2-2	1-1	2-2
Zamora																		
Aliste	Z19	XY	2	2	1	2	2	2	1	1	1	1	2	2	2	2	1	2
	Z22	XY	1	2	1	2	1	2	1	2	2	2	1	1	2	1	2	2
	Z87	XY	1	1	1	2	2	1	1	1	1	2	2	2	2	2	1	1
	Z99	XY	1	2	2	2	2	2	1	1	1	1	2	2	2	2	1	2
	Z154	XY	2	2	2	1	2	2	1	2	2	1	1	2	2	2	2	2
	Z156	XY	2	1	2	2	1	2	1	2	1	2	1	2	2	2	2	2
	Z164	XY	1	2	2	2	1	2	1	2	2	2	1	2	2	2	1	2
	Z213	XY	1	2	2	2	1	2	1	2	1	2	2	2	2	2	2	2
	Z231	XY	1	2	1	2	2	2	1	2	1	2	2	2	1	2	2	2
	Z241	XY	2	1	1	2	1	1	1	1	2	2	2	2	1	2	1	1
	Z246	XY	1	2	1	2	2	2	1	1	2	1	2	2	1	1	2	2
	Z272	XY	2	2	2	2	2	2	1	2	1	2	1	2	2	2	2	2
	Z287	XY	2	2	2	2	1	2	2	2	1	2	2	2	2	1	2	1
	Z304	XY	1	2	1	2	2	2	1	2	1	2	1	2	2	1	1	2
	Z307	XY	2	2	2	2	1	2	1	1	2	1	1	2	2	2	1	2
	Z308	XY	2	2	2	2	1	2	1	1	1	2	2	1	2	2	1	1
	Z309	XY	1	1	2	2	1	2	1	2	2	2	2	2	2	2	2	2
	Z310	XY	2	1	2	2	2	2	1	2	2	2	2	2	2	2	1	1
	Z311	XY	1	2	2	2	1	2	2	2	1	2	2	1	2	2	2	2
	Z315	XY	1	1	2	1	2	2	1	2	2	2	2	2	2	2	2	2
	Z318	XY	1	2	2	2	2	2	1	2	1	1	2	2	1	2	1	2
	Z319	XY	2	2	2	1	2	1	1	2	1	2	1	2	2	2	1	2
	Z320	XY	2	2	2	2	1	2	1	1	2	1	2	1	2	1	2	1
	Z321	XY	1	2	1	2	1	2	1	2	2	2	2	2	2	2	1	2
	Z322	XY	1	1	1	2	1	2	1	2	1	2	2	1	2	2	2	2

Bajo-Duero	Z29	XX	1 - 1	1 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 1	2 - 2	2 - 2	2 - 2	1 - 2	2 - 2	2-2	1-2	2-2	2-2
	Z84	XX	2 - 2	1 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 2	2 - 2	1 - 2	1 - 2	2-2	1-2	1-2	1-2
	Z120	XX	1 - 1	2 - 2	2 - 2	2 - 2	1 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 1	1 - 1	1 - 2	1-2	2-2	1-2	2-2
	Z173	XX	1 - 2	1 - 1	2 - 2	2 - 2	1 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 1	1 - 2	2 - 2	1-2	2-2	2-2	2-2
	Z174	XX	2 - 2	2 - 2	2 - 2	1 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 2	1 - 2	2 - 2	1 - 2	2-2	2-2	2-2	1-2
	Z218	XX	1 - 1	2 - 2	2 - 2	1 - 2	1 - 1	2 - 2	1 - 1	2 - 2	1 - 1	1 - 1	2 - 2	2 - 2	1-2	1-2	1-2	1-2
	Z223	XX	2 - 2	1 - 2	1 - 2	1 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1-1	1-2	1-2	1-2
	Z228	XX	1 - 1	2 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 1	2 - 2	2 - 2	1 - 2	2 - 2	2 - 2	2-2	2-2	1-2	2-2
	Z234	XX	1 - 1	1 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 2	2 - 2	1 - 1	1 - 1	1 - 2	2 - 2	2-2	1-1	2-2	2-2
	Z236	XX	1 - 1	1 - 2	2 - 2	2 - 2	1 - 1	1 - 2	1 - 1	2 - 2	1 - 1	2 - 2	2 - 2	1 - 2	1-2	2-2	1-1	1-2
	Z237	XX	1 - 2	2 - 2	1 - 2	1 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 2	2 - 2	2 - 2	2 - 2	1-2	2-2	2-2	1-2
	Z244	XX	1 - 1	1 - 2	1 - 2	1 - 1	1 - 1	1 - 2	1 - 1	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2	2-2	1-2	1-1	2-2
	Z245	XX	1 - 1	2 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1 - 2	1 - 1	2-2	2-2	1-2	2-2
	Z247	XX	1 - 1	2 - 2	1 - 2	2 - 2	1 - 1	1 - 2	1 - 1	1 - 1	1 - 2	1 - 1	2 - 2	1 - 2	2-2	2-2	1-1	2-2
	Z8	XY	2	2	2	2	1	2	1	1	1	1	1	1	2	1	1	2
	Z34	XY	1	1	2	2	2	2	1	2	2	2	2	2	2	2	2	2
	Z67	XY	1	1	2	2	1	2	1	2	1	2	2	2	1	2	2	1
	Z115	XY	1	2	2	2	1	2	1	2	2	2	2	2	1	2	2	2
	Z131	XY	1	2	1	2	1	2	1	2	2	2	2	2	2	2	2	2
	Z151	XY	2	1	2	2	2	2	1	1	2	2	1	2	2	2	2	2
	Z159	XY	1	2	1	2	1	2	1	1	1	2	1	2	1	2	1	2
	Z206	XY	1	2	2	2	2	2	1	2	1	1	2	1	2	2	2	2
	Z222	XY	2	2	2	1	1	2	1	2	1	2	2	2	2	1	2	2
	Z357	XY	1	2	2	1	2	2	1	1	2	2	1	2	1	2	2	1
	Z362	XY	1	2	1	2	1	2	1	1	1	1	2	1	2	2	1	2
	Z364	XY	1	1	2	2	1	2	1	2	1	1	1	1	2	2	2	2
	Z2	XX	1 - 1	2 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 1	1 - 1	1 - 1	2 - 2	1-2	1-2	2-2	2-2
	Z18	XX	1 - 1	1 - 1	1 - 2	1 - 2	1 - 1	2 - 2	1 - 1	2 - 2	1 - 1	2 - 2	2 - 2	2 - 2	2-2	2-2	1-2	2-2
	Z21	XX	1 - 2	1 - 2	2 - 2	1 - 2	1 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 2	1 - 2	2-2	1-2	2-2	2-2
	Z31	XX	1 - 1	2 - 2	1 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1 - 1	1 - 1	2-2	1-2	1-1	2-2

Benavente	Z37	XX	1 - 2	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	1 - 2	1 - 2	2-2	2-2	1-2	2-2
	Z53	XX	1 - 2	2 - 2	2 - 2	2 - 2	1 - 2	2 - 2	1 - 2	1 - 1	1 - 1	1 - 2	1 - 1	2 - 2	1-2	2-2	2-2	2-2
	Z66	XX	1 - 1	1 - 1	1 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 1	1 - 1	2-2	1-2	1-1	2-2
	Z90	XX	1 - 1	2 - 2	2 - 2	1 - 1	1 - 1	2 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 2	1 - 2	2-2	2-2	1-2	2-2
	Z96	XX	1 - 2	1 - 2	2 - 2	1 - 2	1 - 1	1 - 2	1 - 1	2 - 2	1 - 2	2 - 2	1 - 2	2 - 2	2-2	1-2	1-2	1-2
	Z108	XX	1 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	2 - 2	1 - 1	2-2	2-2	2-2	2-2
	Z126	XX	1 - 2	2 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 2	1 - 1	2-2	1-1	1-1	2-2
	Z178	XX	1 - 1	2 - 2	1 - 1	2 - 2	1 - 2	2 - 2	1 - 1	1 - 2	2 - 2	2 - 2	1 - 2	1 - 1	2-2	1-1	1-1	2-2
	Z184	XX	1 - 1	2 - 2	2 - 2	2 - 2	2 - 2	2 - 2	1 - 2	1 - 2	1 - 1	1 - 1	1 - 2	1 - 2	1-2	1-2	2-2	2-2
	Z224	XX	1 - 2	2 - 2	2 - 2	2 - 2	1 - 1	1 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1-2	1-2	1-2	2-2
	Z260	XX	1 - 2	1 - 2	2 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 2	2 - 2	2 - 2	1 - 2	1-2	1-2	1-2	2-2
	Z7	XY	2	2	2	2	2	2	1	1	1	1	2	2	1	2	1	1
	Z70	XY	1	2	2	2	1	2	1	1	1	2	2	2	2	2	1	2
	Z111	XY	2	1	2	1	1	2	2	1	1	2	2	1	2	2	1	2
	Z132	XY	1	2	2	2	2	1	1	2	1	2	1	1	2	2	2	2
	Z210	XY	1	1	1	1	1	2	2	1	1	1	2	1	2	2	2	1
	Z230	XY	1	2	2	2	1	1	1	1	1	1	1	2	2	2	1	2
	Z281	XY	1	2	2	2	1	1	1	1	1	1	2	2	2	2	2	2
	Z327	XY	2	2	2	2	1	1	2	2	1	2	2	2	2	2	2	2
	Z328	XY	2	2	2	1	1	1	1	1	1	1	1	2	2	1	1	2
	Z332	XY	2	2	2	2	1	2	1	2	2	2	2	1	2	2	2	2
	Z333	XY	1	2	2	2	1	2	1	1	1	1	2	2	1	1	2	2
	Z334	XY	2	2	1	1	1	2	2	1	2	1	2	2	2	2	1	2
	Z337	XY	2	2	2	2	1	2	1	2	2	1	2	2	2	2	2	1
	Z338	XY	1	2	2	2	1	2	1	1	1	2	2	2	2	2	2	2
	Z339	XY	1	1	2	2	1	1	1	1	2	2	2	2	2	2	2	2
	Z340	XY	1	2	2	2	1	2	1	2	1	2	2	1	1	2	1	2
	Z341	XY	1	1	1	2	1	2	1	2	1	2	1	2	1	2	2	2
	Z344	XY	2	2	2	1	2	2	1	1	2	1	1	2	2	2	1	2
	Z356	XY	1	2	2	2	2	1	1	1	2	2	1	1	2	2	1	2

Campos-Pan	Z383	XY	1	2	1	2	2	2	1	1	1	1	2	1	2	2	1	1
	Z3	XX	1 - 2	2 - 2	2 - 2	1 - 1	1 - 1	2 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1 - 1	2 - 2	2 - 2	1 - 2	2 - 2	2 - 2
	Z11	XX	1 - 1	2 - 2	2 - 2	2 - 2	1 - 2	2 - 2	1 - 1	1 - 2	1 - 1	1 - 2	2 - 2	1 - 1	2 - 2	1 - 2	2 - 2	2 - 2
	Z16	XX	1 - 2	2 - 2	2 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 1	2 - 2	1 - 1	2 - 2
	Z137	XX	1 - 1	1 - 2	2 - 2	1 - 1	1 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 2	2 - 2
	Z141	XX	1 - 2	2 - 2	1 - 2	2 - 2	1 - 2	1 - 2	1 - 1	1 - 2	1 - 2	2 - 2	2 - 2	2 - 2	2 - 2	1 - 2	2 - 2	2 - 2
	Z179	XX	1 - 1	2 - 2	1 - 2	2 - 2	1 - 2	2 - 2	1 - 1	1 - 1	1 - 2	1 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 2	2 - 2
	Z182	XX	1 - 1	1 - 2	2 - 2	1 - 1	1 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2	1 - 2	2 - 2
	Z201	XX	1 - 2	2 - 2	1 - 1	2 - 2	1 - 1	2 - 2	1 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 2	1 - 2	2 - 2
	Z217	XX	1 - 2	1 - 2	1 - 2	1 - 2	1 - 2	2 - 2	1 - 1	1 - 2	1 - 2	2 - 2	2 - 2	2 - 2	2 - 2	2 - 2	1 - 2	1 - 2
	Z227	XX	1 - 2	2 - 2	1 - 2	2 - 2	1 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 2	1 - 2	2 - 2
	Z229	XX	1 - 2	2 - 2	1 - 2	1 - 2	1 - 2	2 - 2	1 - 1	1 - 2	1 - 1	2 - 2	1 - 2	1 - 2	2 - 2	2 - 2	1 - 2	2 - 2
	Z251	XX	1 - 2	2 - 2	1 - 2	1 - 2	1 - 1	1 - 2	1 - 1	2 - 2	1 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 2	2 - 2	2 - 2
	Z253	XX	1 - 1	2 - 2	2 - 2	1 - 1	1 - 1	2 - 2	1 - 1	1 - 2	1 - 1	2 - 2	1 - 2	1 - 2	1 - 2	2 - 2	1 - 1	2 - 2
	Z263	XX	1 - 1	2 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 1	2 - 2	1 - 2	1 - 1	2 - 2	2 - 2	2 - 2	1 - 2	2 - 2	1 - 2
	Z265	XX	1 - 1	2 - 2	1 - 2	1 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 1	2 - 2	2 - 2	2 - 2	1 - 2	2 - 2
	Z268	XX	1 - 1	2 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 2	1 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 2	1 - 2
	Z273	XX	1 - 1	2 - 2	2 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 2	2 - 2	2 - 2	1 - 2	1 - 2	2 - 2	2 - 2	2 - 2
	Z38	XY	2	2	2	2	1	2	1	1	1	2	2	2	2	2	1	1
	Z41	XY	2	2	2	2	2	1	1	2	1	2	2	2	1	2	1	1
	Z44	XY	1	1	2	2	1	2	1	1	1	2	2	2	1	2	2	2
	Z46	XY	1	2	2	2	2	2	1	1	1	2	1	2	2	2	1	2
	Z49	XY	1	1	2	1	1	2	1	2	1	2	2	1	2	2	2	2
	Z54	XY	2	2	2	2	1	2	1	2	1	1	2	2	2	2	2	2
	Z79	XY	1	1	2	2	1	2	1	2	1	2	2	2	2	1	2	1
	Z106	XY	1	2	2	2	1	2	1	2	1	1	2	2	1	2	2	2
	Z114	XY	1	2	2	2	1	2	1	2	1	1	1	1	2	1	2	2
	Z127	XY	1	1	2	2	1	2	1	1	2	2	2	1	2	1	1	2
	Z130	XY	1	2	2	2	1	2	1	2	1	1	1	2	2	1	2	2
	Z160	XY	1	1	2	2	2	2	1	2	1	1	2	2	2	2	1	1

	Z176	XY	2	1	2	2	2	2	1	1	2	2	1	2	2	2	1	2
	Z177	XY	2	1	1	2	1	2	1	2	1	1	1	2	2	2	1	2
	Z198	XY	1	2	2	2	1	2	1	2	1	2	1	1	2	2	2	2
	Z249	XY	2	2	2	1	1	2	1	2	2	2	1	2	2	2	2	2
	Z264	XY	1	2	2	2	1	2	1	2	2	1	2	1	2	2	1	2
	Z342	XY	2	1	2	2	2	2	2	2	1	2	2	1	2	2	2	2
	Z373	XY	1	2	2	2	1	1	2	2	1	2	2	2	2	2	2	2
	Z378	XY	1	2	2	1	1	2	1	2	1	2	1	2	2	2	1	2
	Z379	XY	1	2	2	1	2	1	1	2	1	1	2	2	2	2	2	1
	Z380	XY	2	2	1	2	1	2	1	2	2	2	2	2	2	2	1	2
	Z381	XY	2	1	2	2	1	2	1	2	1	1	1	1	2	1	1	2
	Z382	XY	1	2	2	2	2	2	1	2	1	1	2	2	1	2	1	2
	Z384	XY	1	2	2	2	2	2	1	2	2	1	2	2	2	1	1	2
	Z391	XY	1	2	2	2	1	2	2	2	2	2	1	2	2	2	1	2
	Z399	XY	2	2	2	1	1	1	1	1	2	2	2	1	1	2	2	2
	Z9	XX	1 - 2	1 - 2	2 - 2	1 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 2	2 - 2	1 - 1	2 - 2	2 - 2	2 - 2	1 - 2	2 - 2
	Z10	XX	1 - 1	2 - 2	2 - 2	2 - 2	1 - 2	1 - 2	1 - 1	2 - 2	1 - 2	2 - 2	1 - 2	2 - 2	2 - 2	2 - 2	1 - 2	2 - 2
	Z30	XX	2 - 2	2 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 2	1 - 2	2 - 2	? - ?
	Z56	XX	1 - 1	2 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2	2 - 2	1 - 1	1 - 2
	Z101	XX	2 - 2	1 - 1	1 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	2 - 2	2 - 2	2 - 2	2 - 2	2 - 2	2 - 2	2 - 2	1 - 2
	Z110	XX	1 - 2	1 - 2	2 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 2	1 - 2	2 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2
	Z125	XX	2 - 2	2 - 2	2 - 2	2 - 2	1 - 1	1 - 2	1 - 1	1 - 2	2 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 1	1 - 2	1 - 2
	Z133	XX	1 - 1	2 - 2	2 - 2	1 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 2	2 - 2	2 - 2	2 - 2	2 - 2	2 - 2
	Z134	XX	1 - 2	1 - 2	2 - 2	2 - 2	1 - 2	1 - 2	1 - 1	1 - 1	1 - 2	2 - 2	1 - 2	2 - 2	1 - 2	2 - 2	2 - 2	2 - 2
	Z138	XX	1 - 1	1 - 2	2 - 2	1 - 2	2 - 2	2 - 2	1 - 2	1 - 2	1 - 1	1 - 2	2 - 2	2 - 2	2 - 2	2 - 2	2 - 2	2 - 2
	Z142	XX	1 - 1	1 - 2	2 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 1	2 - 2	1 - 2	2 - 2	1 - 2	1 - 2	1 - 1	2 - 2
	Z145	XX	1 - 1	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 2	1 - 1	2 - 2	2 - 2	2 - 2	2 - 2
	Z146	XX	1 - 2	2 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 1	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2	2 - 2	1 - 2	2 - 2
	Z163	XX	1 - 2	1 - 2	1 - 2	1 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	1 - 2	2 - 2
	Z165	XX	1 - 2	1 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 1	2 - 2	1 - 2	2 - 2	1 - 2	2 - 2	2 - 2	2 - 2	1 - 1	2 - 2

Sanabria	Z175	XX	1 - 1	1 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1-2	2-2	1-1	1-2
	Z180	XX	1 - 2	2 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 1	2 - 2	1 - 2	1 - 2	1-2	2-2	2-2	1-2
	Z185	XX	1 - 1	1 - 2	1 - 1	2 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 1	1 - 2	2 - 2	1 - 2	1-2	2-2	1-1	2-2
	Z203	XX	1 - 1	1 - 2	2 - 2	2 - 2	1 - 1	1 - 2	1 - 1	1 - 1	2 - 2	2 - 2	2 - 2	1 - 1	2-2	2-2	1-2	2-2
	Z205	XX	1 - 1	2 - 2	2 - 2	1 - 1	1 - 2	2 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 2	1 - 2	2-2	1-2	2-2	2-2
	Z69	XY	1	1	1	2	1	1	1	2	2	2	1	2	2	2	2	2
	Z168	XY	1	2	2	2	1	2	1	2	1	2	1	2	2	2	2	2
	Z186	XY	2	2	1	1	1	2	1	1	2	1	2	2	2	2	2	2
	Z239	XY	1	2	2	1	1	2	1	2	1	2	2	2	2	2	1	2
	Z242	XY	1	1	2	2	1	2	1	2	2	2	1	2	2	2	2	2
	Z271	XY	1	1	2	2	2	2	1	1	1	2	1	2	2	1	2	2
	Z274	XY	1	2	2	1	1	2	1	2	1	1	1	1	2	2	2	2
	Z275	XY	2	1	1	1	2	2	1	2	1	2	2	1	2	2	2	2
	Z286	XY	1	2	2	2	2	2	1	2	1	2	2	2	2	2	2	2
	Z377	XY	1	1	2	1	2	2	1	1	1	1	2	2	1	2	2	2
	Z72	XX	1 - 2	1 - 2	2 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 2	1 - 1	2 - 2	1 - 2	2-2	1-2	1-2	2-2
	Z181	XX	1 - 1	1 - 1	2 - 2	2 - 2	1 - 2	2 - 2	1 - 1	1 - 2	1 - 1	1 - 2	2 - 2	1 - 1	1-2	2-2	1-2	2-2
	Z238	XX	1 - 1	2 - 2	1 - 2	1 - 1	1 - 1	2 - 2	1 - 1	1 - 1	1 - 1	2 - 2	2 - 2	1 - 2	2-2	2-2	1-2	1-2
	Z270	XX	1 - 1	1 - 2	2 - 2	2 - 2	1 - 2	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2	1 - 2	1 - 2	2-2	1-2	1-2	2-2
Sayago	Z278	XX	1 - 1	2 - 2	2 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 1	2 - 2	2 - 2	1 - 2	2-2	2-2	2-2	1-1
	Z279	XX	1 - 1	1 - 2	2 - 2	2 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 2	1 - 2	2 - 2	2 - 2	2-2	2-2	2-2	1-2
	Z282	XX	1 - 1	1 - 1	1 - 2	1 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 2	2 - 2	2 - 2	1 - 2	2-2	1-2	2-2	1-2
	Z283	XX	2 - 2	2 - 2	2 - 2	2 - 2	1 - 2	1 - 2	1 - 2	2 - 2	2 - 2	1 - 2	1 - 2	1 - 2	2-2	2-2	1-2	2-2
	Z285	XX	1 - 2	1 - 2	1 - 2	2 - 2	1 - 2	1 - 2	1 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 1	1-2	2-2	1-1	2-2
	Z289	XX	1 - 1	2 - 2	2 - 2	1 - 2	2 - 2	2 - 2	1 - 2	2 - 2	2 - 2	2 - 2	2 - 2	1 - 2	2-2	1-2	1-2	1-2
	Z6	XY	1	1	2	2	2	2	1	1	1	2	1	2	2	2	2	2
	Z73	XY	1	2	2	2	1	1	1	1	1	2	2	1	1	1	1	2
	Z88	XY	2	1	2	1	1	2	1	1	2	2	1	1	1	2	2	2
	Z118	XY	2	2	1	2	1	2	1	1	1	2	2	1	2	2	2	2
	Z119	XY	1	2	2	2	2	1	1	1	1	2	2	2	2	2	2	2

	Z123	XY	1	1	2	2	1	2	1	2	2	2	1	1	2	2	1	2
	Z124	XY	1	1	2	2	2	2	1	2	1	1	2	2	1	2	1	1
	Z158	XY	2	2	2	2	1	1	1	2	1	1	2	1	2	2	1	2
	Z189	XY	1	2	2	1	2	2	1	1	1	2	2	1	1	2	1	2
	Z204	XY	1	1	2	2	2	2	1	2	2	2	1	1	2	2	1	2
	Z252	XY	1	2	2	2	1	1	1	1	1	2	2	2	2	1	1	2
	Z257	XY	1	2	2	2	1	2	1	2	2	2	2	2	1	1	2	2
	Z276	XY	1	2	2	2	1	2	2	2	1	2	2	2	2	1	1	2
	Z293	XY	2	2	2	2	1	2	1	1	1	1	2	2	2	2	2	1
	Z294	XY	1	1	1	2	1	2	1	1	2	1	2	2	2	2	1	2
	Z295	XY	1	2	2	2	1	2	1	2	2	2	1	2	2	2	1	2
	Z296	XY	2	2	2	2	2	1	1	1	2	2	1	2	2	2	1	2
	Z297	XY	2	2	2	2	2	2	1	1	1	2	1	1	2	2	2	2
	Z300	XY	1	2	2	2	2	2	1	2	2	2	2	1	2	2	1	1
	Z302	XY	1	1	2	2	1	2	1	1	1	1	2	1	1	2	1	2
	Z303	XY	1	2	1	2	1	1	1	2	1	2	2	2	1	2	1	1
	Z86	XX	1 - 2	1 - 2	2 - 2	1 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 1	2 - 2	1 - 1	1 - 2	2-2	2-2	1-2	2-2
	Z98	XX	1 - 2	1 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 2	2-2	2-2	1-2	2-2
	Z139	XX	1 - 2	2 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 2	2 - 2	1 - 2	1 - 2	1 - 2	1 - 1	1-2	1-2	1-2	2-2
	Z144	XX	1 - 1	2 - 2	2 - 2	2 - 2	1 - 2	2 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 1	2 - 2	2-2	1-2	2-2	2-2
	Z153	XX	1 - 2	2 - 2	2 - 2	1 - 1	1 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 2	2 - 2	2 - 2	1-2	2-2	1-2	1-2
	Z166	XX	1 - 2	1 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 1	2 - 2	1 - 2	1 - 2	1 - 1	1 - 2	2-2	2-2	1-1	2-2
	Z209	XX	1 - 1	2 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 2	2 - 2	2-2	2-2	2-2	2-2
	Z220	XX	1 - 2	1 - 2	2 - 2	1 - 2	1 - 1	2 - 2	1 - 1	2 - 2	1 - 1	2 - 2	1 - 2	1 - 1	2-2	2-2	1-2	2-2
	Z256	XX	2 - 2	1 - 2	2 - 2	1 - 2	1 - 2	1 - 1	1 - 1	1 - 2	1 - 2	2 - 2	1 - 1	1 - 2	2-2	2-2	2-2	1-2
	Z288	XX	1 - 2	2 - 2	2 - 2	2 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 1	2 - 2	1 - 2	2 - 2	1-2	2-2	1-2	2-2

Sample		MID 2089	MID 3774	MID 3760	MID 3701	MID 2612	MID 1839	MID 3754	MID 111	MID 2652	MID 1511	MID 2692	MID 357	MID 356	MID 243	MID 3727	MID 3753
Miranda do Douro	MD 02	2	2	2	1	2	1	2	2	2	1	1	1	1	1	2	2
	MD 07	1	2	1	2	1	1	2	1	1	1	1	2	2	1	2	2
	MD 09	1	2	2	1	1	1	2	2	1	2	1	1	1	1	2	2
	MD 11	2	2	1	2	2	1	1	1	2	1	1	2	2	1	1	2
	MD 19	2	2	1	2	2	1	2	2	1	1	1	2	2	2	2	2
	MD 25	2	2	1	2	1	1	2	1	2	2	1	2	2	1	2	2
	MD 50	2	2	2	2	2	2	1	2	1	1	2	2	2	1	2	2
	MD 51	2	2	1	2	2	1	2	1	2	1	1	1	1	2	2	2
	MD 58	1	2	2	2	1	1	2	1	1	1	1	1	1	1	2	2
	MD 59	2	2	1	2	2	1	2	1	1	2	1	2	2	1	2	2
	MD 61	2	2	1	1	2	1	2	1	1	1	1	2	2	1	2	2
	MD 64	2	1	1	2	2	1	2	1	1	1	1	2	2	1	2	2
	MD 73	2	1	1	2	1	1	2	2	1	1	1	2	2	2	1	2
	MD 93	2	2	1	1	1	1	2	2	1	1	1	2	2	1	2	1
	MD 94	2	2	1	2	2	2	2	2	2	1	2	2	2	1	2	2
	MD L05	2	2	1	2	2	1	2	2	1	1	1	2	2	1	2	2
	MD L09	2	2	2	2	2	1	2	2	1	1	2	2	1	1	2	2
	MD L11	2	2	1	2	1	1	2	1	2	2	2	1	1	1	1	2
	MD L12	1	2	1	2	2	1	2	2	1	1	1	2	2	1	2	2
	MD L22	2	2	1	1	1	1	2	1	1	1	1	2	2	1	2	2
	MD L25	2	1	1	1	2	2	2	2	1	1	1	2	2	2	2	2
	MD L26	2	1	1	2	2	2	2	1	2	2	1	2	2	2	2	2
	MD L29	2	1	1	2	1	1	2	2	?	2	1	2	2	2	2	2
	MD L32	2	2	1	1	2	2	1	1	2	2	2	2	2	1	2	2
	MD L34	1	2	1	2	2	1	2	1	1	1	1	2	2	2	2	2
	MD L35	2	2	1	1	2	1	2	1	1	1	2	1	1	2	2	2
	MD L38	2	2	1	2	2	1	2	1	2	2	2	1	1	1	2	2
	MD L39	1	2	1	1	2	1	2	?	1	1	1	1	1	1	2	2

	MD L46	2	2	1	2	1	1	2	2	?	2	1	2	2	1	2	2
	MD L49	2	2	2	2	2	1	2	2	1	2	1	2	2	1	2	2
	MD L56	2	1	2	2	1	1	2	2	1	1	1	2	?	1	1	2
	MD L57	2	1	1	2	1	1	2	2	1	1	1	2	2	1	2	2
	MD L60	1	1	2	2	2	1	2	2	1	1	1	2	2	1	2	2
	MD S1	2	2	2	2	1	1	2	1	2	1	1	1	1	1	2	2
	MD S2	1	2	1	1	2	2	1	1	2	1	1	2	?	1	2	2
	MD S5	2	2	1	2	1	2	2	1	1	1	1	1	1	1	2	2
	MD S7	1	2	1	2	2	1	2	1	1	1	1	1	1	1	2	2
	MD S8	2	2	1	1	1	1	2	2	1	1	1	1	2	2	2	2
	MD S9	2	2	2	1	2	1	2	2	1	2	1	2	2	1	2	2
	MD S10	2	2	2	2	2	1	2	1	2	1	1	1	1	2	2	1
	MD S13	1	2	2	2	1	1	2	2	1	1	2	2	1	1	2	2
	MD S16	2	2	1	2	2	1	2	2	1	1	2	1	1	1	2	2
	MD S24	2	2	2	2	1	1	2	1	1	1	2	2	2	1	2	2
	MD S27	1	2	1	2	2	1	2	1	1	1	2	2	2	1	2	2
	MD S30	2	2	1	1	2	2	2	2	2	1	1	1	?	1	2	2
	MD S31	1	2	1	2	2	1	1	2	2	1	1	1	1	1	2	2
	MD S32	2	2	2	2	1	1	2	1	1	1	1	2	2	1	2	2
	MD S33	2	2	2	1	1	1	2	1	1	2	2	1	1	2	1	2
	MD S34	2	1	1	2	2	1	2	2	1	1	1	2	2	1	2	2
	MD S35	1	2	1	2	2	1	1	1	1	1	1	2	2	1	2	2
	MD S36	2	2	1	1	2	1	2	1	1	1	2	2	2	1	2	2
	MD X3	2	2	1	2	1	?	2	1	2	1	?	2	?	1	1	2
	MD X6	2	2	1	2	2	2	2	2	1	2	1	2	2	1	2	2
	MD X7	2	2	1	2	2	1	2	2	1	1	1	2	2	1	2	2
	MD 4	1 - 2	2 - 2	1 - 1	1 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 2	1 - 2	2 - 2	2 - 2	2 - 2	1 - 1	2 - 2	2 - 2
	MD 5	1 - 2	1 - 2	1 - 2	1 - 2	2 - 2	1 - 2	1 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 1	1 - 1	1 - 2	2 - 2	2 - 2
	MD 6	1 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 1	1 - 1	1 - 2	1 - 2	1 - 1	2 - 2	2 - 2
	MD 8	1 - 2	1 - 1	1 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 2	1 - 1	2 - 2	2 - 2	1 - 1	2 - 2	2 - 2

	MD 10	1 - 2	1 - 2	1 - 2	2 - 2	2 - 2	1 - 1	1 - 2	2 - 2	1 - 1	1 - 2	2 - 2	2 - 2	2-2	1-1	2-2	1-2
	MD 12	2 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 2	1 - 2	1 - 2	1 - 1	1 - 1	1-1	1-1	2-2	2-2
	MD 15	2 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1 - 1	2 - 2	1 - 1	1 - 2	2 - 2	2 - 2	2 - 2	2-2	1-2	1-2	2-2
	MD 17	2 - 2	2 - 2	1 - 2	1 - 2	1 - 1	1 - 1	2 - 2	1 - 2	1 - 2	1 - 1	1 - 2	2 - 2	2-2	1-2	2-2	2-2
	MD 18	1 - 2	2 - 2	1 - 1	1 - 2	2 - 2	1 - 2	1 - 2	1 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1-1	1-1	2-2	2-2
	MD 20	2 - 2	2 - 2	1 - 1	1 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 2	1 - 1	2 - 2	2-2	1-1	2-2	2-2
	MD 21	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 2	1 - 1	2 - 2	2-2	1-2	2-2	2-2
	MD 26	1 - 1	1 - 2	1 - 1	2 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 1	2 - 2	2 - 2	2 - 2	1-2	1-1	2-2	2-2
	MD 27	1 - 2	2 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 1	1 - 1	1 - 2	1 - 2	1 - 1	2 - 2	1-2	1-1	2-2	2-2
	MD 48	1 - 2	2 - 2	2 - 2	2 - 2	1 - 1	1 - 1	2 - 2	2 - 2	1 - 2	1 - 2	1 - 2	2 - 2	2-2	1-1	2-2	2-2
	MD 54	2 - 2	2 - 2	1 - 1	1 - 1	1 - 1	1 - 1	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 2	1-2	1-1	1-2	2-2
	MD 55	2 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1 - 1	1 - 2	1 - 2	1-2	1-1	1-2	2-2
	MD 56	2 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 2	2 - 2	1 - 1	1 - 1	1 - 2	1 - 1	1 - 2	1-2	1-1	2-2	1-2
	MD 57	2 - 2	1 - 2	1 - 2	1 - 1	1 - 1	1 - 1	2 - 2	2 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1-2	1-1	2-2	2-2
	MD 60	2 - 2	2 - 2	1 - 2	1 - 2	1 - 2	1 - 2	2 - 2	2 - 2	1 - 2	1 - 1	1 - 2	2 - 2	2-2	1-1	2-2	2-2
	MD 62	1 - 2	2 - 2	1 - 1	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1-1	1-1	2-2	2-2
	MD 63	1 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1 - 1	1 - 2	1-2	1-1	2-2	2-2
	MD 65	2 - 2	2 - 2	1 - 2	2 - 2	1 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1 - 1	1 - 1	2 - 2	2-2	1-1	2-2	1-2
	MD 66	1 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 1	1 - 1	2 - 2	2-2	1-1	2-2	2-2
	MD 67	1 - 1	1 - 2	1 - 1	1 - 2	1 - 1	1 - 1	2 - 2	1 - 2	1 - 1	2 - 2	2 - 2	2 - 2	2-2	1-1	2-2	2-2
	MD 69	2 - 2	1 - 2	1 - 2	2 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1-2	1-2	2-2	2-2
	MD 70	2 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 2	1 - 2	1 - 1	1 - 1	1 - 1	1 - 1	1-1	1-1	2-2	2-2
	MD 71	2 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 1	1 - 2	1 - 1	2 - 2	2-2	1-1	2-2	2-2
	MD 72	2 - 2	2 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1-2	1-2	2-2	2-2
	MD 74	1 - 1	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1-2	1-2	2-2	1-2
	MD 89	2 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1-2	1-2	2-2	2-2
	MD 90	1 - 1	2 - 2	1 - 2	1 - 1	1 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 1	1 - 2	2 - 2	1-2	1-1	2-2	2-2
	MD L01	1 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 1	2 - 2	1 - 1	1 - 2	1-1	1-1	2-2	2-2
	MD L03	2 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 2	1 - 1	1 - 2	1-2	1-1	2-2	1-1
	MD L06	2 - 2	1 - 2	1 - 2	2 - 2	1 - 1	1 - 1	1 - 2	1 - 1	1 - 1	1 - 1	1 - 1	1 - 2	1-2	1-1	1-2	2-2

	MD L08	1 - 1	2 - 2	1 - 1	1 - 1	2 - 2	1 - 2	2 - 2	2 - 2	1 - 1	1 - 1	1 - 1	2 - 2	1-2	1-1	2-2	2-2
	MD L10	1 - 2	2 - 2	1 - 1	1 - 2	1 - 2	2 - 2	2 - 2	2 - 2	1 - 2	1 - 1	1 - 1	2 - 2	1-2	1-1	2-2	1-2
	MD L13	1 - 2	1 - 2	1 - 2	1 - 2	1 - 1	1 - 2	2 - 2	1 - 2	1 - 1	1 - 1	1 - 1	2 - 2	2-2	1-1	2-2	2-2
	MD L14	2 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 2	2 - 2	1 - 2	1 - 1	1 - 1	1 - 2	1-2	1-1	1-2	2-2
	MD L15	2 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 1	2 - 2	2 - 2	2 - 2	2 - 2	1 - 1	2 - 2	2-2	1-1	2-2	2-2
	MD L16	1 - 2	1 - 2	1 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1 - 1	1 - 1	1 - 2	1 - 1	1 - 2	1-2	1-1	1-2	2-2
	MD L17	1 - 2	2 - 2	1 - 1	1 - 1	1 - 2	1 - 1	2 - 2	2 - 2	1 - 1	1 - 1	1 - 1	2 - 2	1-1	1-1	1-2	2-2
	MD L20	1 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 2	2 - 2	1 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1-2	1-1	2-2	2-2
	MD L23	1 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1-1	1-2	2-2	2-2
	MD L28	1 - 2	2 - 2	1 - 1	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	1 - 1	2 - 2	2 - 2	2 - 2	2-2	1-2	1-2	2-2
	MD L36	1 - 2	1 - 2	1 - 2	1 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 2	1 - 1	1 - 1	2 - 2	1-2	1-1	2-2	2-2
	MD L37	2 - 2	2 - 2	1 - 1	1 - 2	2 - 2	1 - 2	2 - 2	1 - 1	1 - 1	1 - 2	2 - 2	1 - 2	1-2	1-2	2-2	2-2
	MD L40	1 - 2	2 - 2	1 - 2	1 - 2	1 - 2	1 - 1	2 - 2	2 - 2	1 - 1	1 - 1	1 - 1	1 - 1	1-1	1-1	2-2	2-2
	MD L41	1 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1-2	1-2	2-2	2-2
	MD L42	1 - 2	1 - 2	1 - 2	2 - 2	1 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 1	1 - 1	2 - 2	2-2	1-1	1-2	2-2
	MD L43	2 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 2	1 - 2	1 - 2	1-1	1-2	2-2	2-2
	MD L44	1 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 1	1 - 2	? - ?	1 - 1	1 - 1	1 - 2	1 - 2	1-2	1-1	2-2	1-2
	MD L48	2 - 2	2 - 2	1 - 1	1 - 2	1 - 1	1 - 2	2 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1 - 2	1-2	1-1	2-2	2-2
	MD L51	2 - 2	1 - 2	1 - 1	2 - 2	1 - 1	1 - 1	2 - 2	1 - 1	1 - 2	1 - 1	1 - 1	2 - 2	2-2	1-2	2-2	1-2
	MD L53	2 - 2	2 - 2	2 - 2	2 - 2	1 - 2	? - ?	1 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 2	?-?	1-1	2-2	2-2
	MD L59	1 - 1	2 - 2	1 - 2	1 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 1	1 - 1	1 - 2	?-?	1-2	2-2	1-2
	MD S4	2 - 2	2 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 1	1-1	1-1	2-2	2-2
	MD S11	1 - 2	1 - 1	1 - 1	2 - 2	1 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 2	2 - 2	2 - 2	2-2	1-1	2-2	1-2
	MD S12	1 - 1	1 - 2	1 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 1	1 - 2	2 - 2	2-2	2-2	2-2	1-2
	MD S14	2 - 2	2 - 2	1 - 2	2 - 2	1 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 1	1 - 1	1 - 2	1-1	1-1	2-2	2-2
	MD S15	1 - 2	2 - 2	2 - 2	2 - 2	1 - 1	1 - 1	2 - 2	2 - 2	1 - 2	1 - 2	1 - 2	2 - 2	2-2	1-1	2-2	2-2
	MD S17	1 - 1	1 - 2	1 - 2	1 - 1	2 - 2	1 - 2	2 - 2	1 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1-2	1-1	2-2	2-2
	MD S20	2 - 2	2 - 2	1 - 2	2 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 2	1 - 2	1 - 2	2 - 2	2-2	1-1	1-2	2-2
	MD S21	2 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2	2-2	1-1	1-2	2-2
	MD S26	1 - 1	2 - 2	1 - 1	1 - 2	1 - 1	1 - 1	2 - 2	1 - 2	1 - 1	1 - 1	1 - 1	2 - 2	1-1	1-1	2-2	2-2

	MD S28	1 - 2	2 - 2	1 - 1	1 - 1	2 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 1	1 - 2	2 - 2	2-2	2-2	1-2	2-2
	MD X8	1 - 2	2 - 2	1 - 1	2 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 1	1-1	1-1	2-2	2-2
Zamora																	
Aliste	Z19	2	2	1	2	2	1	1	2	1	1	2	1	1	1	2	2
	Z22	1	1	1	2	1	1	2	1	1	1	2	2	2	1	2	2
	Z87	1	2	1	2	2	2	2	1	1	2	1	2	2	1	2	2
	Z99	2	2	1	1	1	1	1	2	1	1	1	1	1	1	1	2
	Z154	2	2	1	2	1	1	2	1	2	2	1	2	1	1	2	2
	Z156	2	2	1	2	2	1	2	1	1	1	2	2	1	1	2	2
	Z164	2	1	1	1	2	1	2	1	2	1	1	1	1	1	2	2
	Z213	2	2	2	1	2	1	2	1	1	1	1	2	1	2	1	2
	Z231	2	2	1	1	2	1	1	2	1	1	1	2	2	1	1	2
	Z241	1	1	1	2	2	1	2	2	2	1	2	2	2	1	2	2
	Z246	2	2	2	1	2	1	2	1	1	1	1	2	2	1	2	2
	Z272	1	1	1	1	2	?	2	2	1	1	2	2	2	1	2	1
	Z287	2	2	1	2	1	1	2	1	1	1	1	1	1	1	1	2
	Z304	2	2	1	1	2	2	2	1	1	1	2	2	2	1	2	2
	Z307	2	2	1	2	1	1	2	2	2	1	1	2	2	1	2	2
	Z308	2	1	1	1	2	1	2	1	1	1	1	1	1	1	2	2
	Z309	2	2	1	2	1	1	1	2	1	1	1	1	1	2	2	2
	Z310	1	2	2	2	2	1	2	1	1	2	2	2	2	1	2	2
	Z311	2	2	1	1	1	1	2	2	1	1	1	2	2	1	2	2
	Z315	2	2	1	1	1	1	1	2	2	2	1	1	1	1	2	2
	Z318	2	2	1	2	1	1	2	1	2	2	1	2	2	1	2	2
	Z319	1	2	1	2	2	1	2	1	2	2	1	2	2	1	2	2
	Z320	1	2	2	2	2	1	1	1	1	1	1	1	1	2	2	2
	Z321	2	2	2	1	1	1	2	1	1	2	1	1	1	1	2	2
	Z322	2	2	2	2	2	1	2	2	1	1	2	2	2	2	1	2
	Z29	2 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 1	2 - 2	1 - 2	1 - 2	1 - 2	1 - 2	1 - 2	1-2	1-1	1-2	2-2
	Z84	1 - 1	2 - 2	1 - 1	1 - 2	1 - 1	1 - 1	2 - 2	2 - 2	1 - 2	1 - 2	1 - 2	2 - 2	2-2	1-2	2-2	2-2

Bajo-Duero	Z120	1 - 2	2 - 2	1 - 2	1 - 2	1 - 1	1 - 1	1 - 2	1 - 2	1 - 2	1 - 1	1 - 2	2 - 2	1-2	1-1	2-2	2-2
	Z173	2 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1-2	1-1	1-2	2-2
	Z174	1 - 2	2 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1-2	1-1	1-2	2-2
	Z218	1 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 2	1 - 2	1 - 2	1-2	1-1	2-2	2-2
	Z223	1 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 2	1 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1-2	1-2	1-2	2-2
	Z228	2 - 2	2 - 2	1 - 2	1 - 1	1 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 1	1 - 1	2 - 2	2-2	1-1	2-2	2-2
	Z234	1 - 2	2 - 2	1 - 2	1 - 2	1 - 2	1 - 2	2 - 2	2 - 2	1 - 2	1 - 1	1 - 1	1 - 2	1-2	1-2	1-2	2-2
	Z236	1 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 1	2 - 2	1 - 1	1 - 1	1 - 2	1 - 2	1 - 2	1-2	1-1	1-2	1-2
	Z237	1 - 2	1 - 2	1 - 2	2 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 1	1 - 2	1 - 1	2 - 2	2-2	1-2	2-2	2-2
	Z244	2 - 2	1 - 2	1 - 1	1 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 2	1 - 1	2 - 2	1-2	1-2	1-2	2-2
	Z245	2 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 1	1 - 1	1 - 1	2 - 2	2-2	1-2	1-2	2-2
	Z247	2 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 2	1 - 1	1 - 2	2 - 2	2-2	1-2	2-2	2-2
	Z8	1	2	2	1	2	1	2	1	1	1	1	2	2	1	2	2
	Z34	2	2	1	1	2	1	2	1	2	2	1	1	1	1	2	2
	Z67	1	1	1	2	1	1	2	1	2	1	2	2	2	1	2	2
	Z115	1	2	2	2	1	1	1	1	2	2	1	2	2	1	1	2
	Z131	2	2	2	1	2	2	2	1	2	1	1	1	1	1	1	2
	Z151	2	2	1	1	2	1	2	2	1	1	2	2	2	1	2	2
	Z159	1	2	1	2	2	1	2	2	1	1	1	1	1	2	2	2
	Z206	2	2	1	2	1	1	2	2	1	1	1	2	2	1	2	2
	Z222	2	2	1	2	2	1	2	2	1	2	2	2	2	2	2	1
	Z357	1	2	2	2	2	1	2	1	1	2	1	2	2	1	2	2
	Z362	1	1	1	2	2	1	2	1	1	2	1	1	1	1	2	2
	Z364	2	2	1	1	2	1	2	1	1	2	1	2	2	1	1	2
	Z2	1 - 2	2 - 2	2 - 2	1 - 2	1 - 1	1 - 1	2 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1-2	1-2	2-2	1-2
	Z18	2 - 2	2 - 2	1 - 2	1 - 2	1 - 2	1 - 2	2 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1 - 2	1-2	1-1	2-2	2-2
	Z21	1 - 2	2 - 2	1 - 1	1 - 2	1 - 1	1 - 1	2 - 2	1 - 2	1 - 2	1 - 2	1 - 1	1 - 2	1-2	1-1	2-2	2-2
	Z31	1 - 2	2 - 2	1 - 2	2 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1 - 2	2 - 2	2 - 2	1 - 2	1-2	2-2	1-2	2-2
	Z37	2 - 2	2 - 2	1 - 1	1 - 1	1 - 1	1 - 2	2 - 2	1 - 2	1 - 1	1 - 1	1 - 2	1 - 2	1-2	1-1	2-2	2-2
	Z53	1 - 2	1 - 2	1 - 2	2 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 2	1 - 2	2 - 2	1 - 2	1-2	1-1	2-2	2-2

Benavente	Z66	1 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 2	2 - 2	2 - 2	1 - 2	1 - 1	1 - 1	1 - 2	1-2	1-1	2-2	1-2
	Z90	1 - 2	2 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	1-2	1-2	1-2	2-2
	Z96	1 - 2	2 - 2	1 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1-2	1-1	2-2	1-2
	Z108	2 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 2	1 - 2	1 - 2	1 - 2	1-2	2-2	2-2	2-2
	Z126	2 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1-2	1-2	2-2	2-2
	Z178	2 - 2	1 - 2	1 - 1	1 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1-2	1-2	2-2	2-2
	Z184	2 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1-2	1-2	2-2	2-2
	Z224	2 - 2	1 - 2	1 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1-1	1-2	2-2	2-2
	Z260	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	1 - 2	1 - 2	1 - 2	1 - 2	1 - 1	1 - 2	2 - 2	2-2	1-2	1-1	1-2
	Z7	2	2	1	2	2	1	2	2	1	1	1	2	2	1	2	1
	Z70	2	2	1	2	2	1	2	2	1	1	1	2	2	1	2	2
	Z111	2	1	1	2	1	1	2	1	1	1	1	2	2	1	2	2
	Z132	2	2	1	1	2	2	2	2	2	1	2	2	2	2	2	1
	Z210	1	2	1	1	2	2	1	1	1	2	1	2	2	1	2	2
	Z230	2	1	1	1	2	1	2	2	2	2	1	1	1	1	2	2
	Z281	2	2	1	1	2	1	2	2	2	1	2	2	2	1	1	2
	Z327	2	1	1	2	2	1	2	1	1	1	1	2	2	1	2	2
	Z328	1	2	1	2	1	1	2	1	1	1	2	2	2	1	2	2
	Z332	2	2	1	2	2	1	1	1	1	1	1	1	1	1	2	2
	Z333	2	2	1	2	1	1	2	2	2	1	1	2	2	1	2	2
	Z334	2	2	1	2	2	1	2	1	1	2	2	1	1	1	2	2
	Z337	2	2	2	1	2	1	2	1	1	1	1	1	1	1	2	2
	Z338	2	1	2	1	1	2	2	2	1	1	2	2	2	1	2	2
	Z339	2	1	1	2	2	2	2	1	2	1	2	2	2	1	2	2
	Z340	2	2	1	1	1	1	1	2	1	1	1	2	1	2	2	2
	Z341	1	2	1	1	1	2	2	2	1	1	2	2	2	1	2	2
	Z344	2	2	1	2	1	2	2	2	1	2	1	2	2	1	2	2
	Z356	1	2	2	1	2	1	2	2	1	2	2	2	2	1	2	1
	Z383	1	2	1	1	2	1	2	2	2	1	1	2	2	1	2	2
	Z3	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2	2 - 2	1 - 1	1 - 2	1 - 1	1 - 1	2 - 2	2-2	1-2	2-2	2-2

Campos-Pan	Z11	2 - 2	2 - 2	1 - 2	1 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 1	1 - 2	1 - 2	1-2	1-1	2-2	2-2
	Z16	1 - 1	1 - 2	1 - 1	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	1 - 2	1-2	1-2	2-2	2-2
	Z137	2 - 2	1 - 2	1 - 2	2 - 2	1 - 2	1 - 2	1 - 2	2 - 2	1 - 1	1 - 2	2 - 2	2 - 2	1-2	1-1	1-2	1-2
	Z141	2 - 2	1 - 2	1 - 1	1 - 1	2 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 1	1 - 2	1 - 2	1-1	1-1	2-2	2-2
	Z179	2 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 1	1-1	1-1	2-2	2-2
	Z182	1 - 2	1 - 2	1 - 1	1 - 2	2 - 2	1 - 1	1 - 2	2 - 2	1 - 2	1 - 1	1 - 1	2 - 2	2-2	2-2	2-2	2-2
	Z201	2 - 2	1 - 1	1 - 1	1 - 2	1 - 1	2 - 2	1 - 2	1 - 2	1 - 2	1 - 1	1 - 2	2 - 2	1-2	1-2	2-2	2-2
	Z217	1 - 2	1 - 2	1 - 2	2 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 2	1 - 2	1 - 1	1 - 2	1-2	1-1	2-2	2-2
	Z227	2 - 2	2 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1 - 2	1 - 2	1 - 1	1 - 1	1 - 1	2 - 2	1-2	1-1	2-2	2-2
	Z229	2 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 2	2 - 2	1 - 2	1-2	1-2	2-2	1-2
	Z251	1 - 2	2 - 2	1 - 2	2 - 2	1 - 2	1 - 1	1 - 1	1 - 2	1 - 1	1 - 2	1 - 1	2 - 2	2-2	1-2	2-2	2-2
	Z253	1 - 2	2 - 2	1 - 2	2 - 2	2 - 2	1 - 2	1 - 2	1 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1-2	1-2	2-2	2-2
	Z263	1 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 1	2 - 2	1 - 2	2 - 2	1 - 1	1 - 2	2 - 2	1-2	2-2	1-1	2-2
	Z265	2 - 2	1 - 2	1 - 2	2 - 2	1 - 1	1 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 2	1-2	1-1	1-2	2-2
	Z268	1 - 1	2 - 2	1 - 2	1 - 1	1 - 1	1 - 2	1 - 1	2 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1-1	2-2	2-2	2-2
	Z273	2 - 2	1 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 2	1 - 1	1 - 1	1 - 2	1 - 1	2 - 2	1-2	1-1	1-2	2-2
	Z38	1	2	1	2	1	1	2	1	1	1	1	1	1	2	2	2
	Z41	1	2	2	1	1	1	1	2	1	1	1	2	1	1	1	2
	Z44	2	2	1	1	1	1	1	2	1	2	1	2	2	1	2	2
	Z46	1	2	1	2	1	2	2	1	1	1	1	2	2	2	2	2
	Z49	2	2	2	2	2	1	1	1	1	1	1	1	1	1	2	2
	Z54	2	1	2	2	2	1	1	2	1	1	2	1	1	1	2	2
	Z79	1	2	2	1	1	1	1	1	2	1	1	2	2	1	2	2
	Z106	2	2	?	2	1	1	2	1	1	2	1	1	1	1	1	2
	Z114	2	2	1	1	2	1	2	1	1	1	2	1	1	1	2	2
	Z127	2	2	2	2	2	1	2	1	1	1	1	1	1	1	2	2
	Z130	1	2	2	2	1	1	1	2	1	1	1	1	1	1	2	2
	Z160	1	1	1	2	2	2	2	1	2	1	2	1	1	1	1	2
	Z176	1	2	1	2	2	1	1	2	1	2	1	2	1	1	2	2
	Z177	1	2	1	2	2	1	1	2	1	1	1	2	2	1	2	2

	Z198	2	2	1	2	1	1	2	1	1	2	1	2	2	1	2	2
	Z249	2	2	1	2	1	1	2	2	1	1	1	2	2	1	2	2
	Z264	2	2	1	1	1	1	2	1	2	2	2	2	2	1	2	2
	Z342	2	2	1	1	2	1	1	2	1	1	1	2	2	1	1	2
	Z373	2	2	1	2	2	1	2	1	2	1	2	2	2	1	2	1
	Z378	1	2	1	2	1	1	2	2	1	1	2	1	1	1	2	2
	Z379	1	2	1	2	2	1	2	2	1	1	1	2	2	1	2	2
	Z380	2	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2
	Z381	1	2	1	2	1	2	2	1	1	1	1	2	2	1	2	2
	Z382	2	2	1	2	1	1	2	1	1	1	1	2	2	1	2	2
	Z384	2	2	1	1	2	1	1	2	1	1	2	2	2	1	1	2
	Z391	2	2	1	2	2	1	2	1	1	1	1	1	1	1	2	2
	Z399	2	2	1	1	1	1	2	1	1	2	1	2	2	2	1	1
	Z9	1 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 1	1 - 2	1 - 1	2 - 2	2-2	1-1	2-2	2-2
	Z10	1 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1 - 1	1-1	1-2	2-2	2-2
	Z30	? - ?	2 - 2	1 - 2	1 - 2	1 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 1	? - ?	1 - 2	?-?	2-2	2-2	1-2
	Z56	1 - 2	1 - 2	1 - 2	1 - 2	1 - 1	1 - 1	1 - 2	2 - 2	1 - 1	1 - 2	1 - 1	2 - 2	2-2	2-2	1-2	2-2
	Z101	2 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 2	1 - 1	2 - 2	2-2	1-2	2-2	2-2
	Z110	2 - 2	1 - 1	1 - 2	1 - 2	2 - 2	1 - 1	2 - 2	1 - 2	1 - 2	2 - 2	2 - 2	1 - 2	1-2	1-2	2-2	1-2
	Z125	1 - 1	2 - 2	1 - 2	2 - 2	1 - 2	1 - 2	1 - 2	1 - 2	1 - 1	1 - 1	1 - 2	1 - 2	1-2	1-1	2-2	2-2
	Z133	1 - 2	2 - 2	1 - 2	2 - 2	2 - 2	1 - 2	2 - 2	1 - 1	1 - 1	1 - 2	1 - 2	1 - 2	1-2	1-1	2-2	2-2
	Z134	2 - 2	2 - 2	1 - 2	1 - 2	1 - 2	1 - 1	2 - 2	2 - 2	1 - 1	1 - 2	1 - 1	2 - 2	2-2	1-2	2-2	1-2
	Z138	2 - 2	2 - 2	1 - 1	1 - 1	2 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1-2	1-2	2-2	1-2
	Z142	1 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 2	1 - 1	1 - 1	1 - 2	1-2	1-1	2-2	2-2
	Z145	2 - 2	2 - 2	1 - 1	2 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1 - 1	1-2	1-2	2-2	2-2
	Z146	2 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 1	2 - 2	2-2	1-2	2-2	2-2
	Z163	1 - 2	2 - 2	1 - 2	1 - 2	1 - 2	1 - 1	2 - 2	1 - 2	2 - 2	1 - 1	1 - 1	2 - 2	2-2	1-1	2-2	2-2
	Z165	2 - 2	2 - 2	1 - 1	1 - 1	1 - 2	1 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 2	1 - 2	1-2	1-1	2-2	2-2
	Z175	2 - 2	2 - 2	1 - 1	1 - 2	1 - 1	2 - 2	1 - 2	2 - 2	1 - 2	1 - 1	1 - 1	2 - 2	1-2	2-2	2-2	2-2
	Z180	1 - 2	2 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 2	1 - 2	2 - 2	2 - 2	2 - 2	2-2	1-1	2-2	2-2

Sanabria	Z185	2 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 1	1 - 2	2 - 2	2-2	1-1	2-2	1-2
	Z203	1 - 1	1 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 2	1 - 2	1 - 2	1 - 1	1 - 2	2 - 2	2-2	1-1	1-2	2-2
	Z205	2 - 2	2 - 2	1 - 2	1 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1 - 2	1-2	1-1	2-2	2-2
	Z69	1	2	1	1	2	1	2	2	1	2	1	1	1	1	1	2
	Z168	2	1	2	2	2	1	2	2	1	2	2	2	2	1	2	2
	Z186	2	2	1	2	2	1	2	1	1	1	1	2	2	1	2	1
	Z239	2	2	1	1	2	1	2	2	2	1	1	2	2	2	2	2
	Z242	1	2	1	1	2	1	2	1	1	2	1	2	2	1	2	2
	Z271	1	1	2	1	2	1	2	1	1	2	1	2	2	1	2	1
	Z274	1	2	1	2	2	1	2	2	1	1	2	1	1	1	2	2
	Z275	2	2	1	1	2	2	2	1	1	1	2	2	2	1	2	2
	Z286	2	2	2	2	1	1	2	2	1	1	1	1	1	1	2	1
	Z377	2	2	2	2	2	1	2	2	1	1	1	2	2	1	2	1
	Z72	2 - 2	2 - 2	2 - 2	2 - 2	2 - 2	1 - 1	2 - 2	1 - 2	1 - 2	1 - 2	1 - 2	1 - 2	1-1	1-1	1-2	1-2
	Z181	2 - 2	1 - 2	1 - 1	1 - 1	2 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1 - 2	1-2	1-1	2-2	2-2
	Z238	1 - 2	2 - 2	1 - 1	1 - 1	1 - 2	1 - 1	2 - 2	2 - 2	1 - 1	1 - 2	1 - 1	2 - 2	2-2	1-1	2-2	2-2
	Z270	1 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 1	2 - 2	1 - 1	1 - 1	1 - 1	1 - 1	1 - 1	1-1	1-2	2-2	2-2
	Z278	1 - 2	1 - 2	1 - 2	1 - 2	2 - 2	1 - 2	2 - 2	2 - 2	1 - 1	1 - 1	1 - 2	1 - 1	1-1	1-2	2-2	2-2
	Z279	2 - 2	2 - 2	1 - 2	1 - 2	1 - 2	1 - 2	1 - 2	1 - 2	1 - 1	1 - 1	1 - 2	1 - 2	1-2	1-1	2-2	2-2
	Z282	1 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 1	1 - 1	1 - 2	1-2	1-1	2-2	1-2
	Z283	2 - 2	2 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1 - 2	1 - 2	1 - 2	1-1	1-2	2-2	2-2
	Z285	1 - 1	1 - 2	1 - 1	2 - 2	2 - 2	1 - 1	1 - 2	1 - 1	1 - 1	1 - 1	1 - 2	2 - 2	2-2	1-1	2-2	2-2
	Z289	1 - 2	1 - 2	1 - 2	1 - 2	1 - 1	1 - 2	2 - 2	1 - 2	1 - 1	1 - 1	1 - 2	2 - 2	2-2	1-1	2-2	2-2
Sayago	Z6	2	2	1	2	1	1	2	2	2	1	1	2	2	1	2	2
	Z73	1	2	1	2	2	1	2	1	2	2	2	2	2	2	2	2
	Z88	1	1	1	2	1	1	2	1	2	2	1	2	2	1	2	2
	Z118	2	2	1	1	2	1	2	1	1	1	1	2	2	1	2	2
	Z119	2	2	1	2	2	1	2	1	2	1	1	2	2	1	2	2
	Z123	2	2	2	1	2	1	2	1	1	2	2	2	2	1	2	2
	Z124	1	2	2	2	1	1	2	1	1	1	2	2	2	2	2	2

	Z158	2	2	1	2	2	1	2	1	1	2	1	1	1	2	2	2
	Z189	2	2	1	2	1	1	2	1	1	1	1	2	2	1	2	2
	Z204	2	2	1	2	2	1	2	1	1	1	1	2	2	1	2	1
	Z252	2	2	2	1	2	1	2	1	1	1	1	1	1	1	2	2
	Z257	2	2	1	2	1	1	2	1	2	1	1	1	1	2	2	2
	Z276	2	2	1	1	2	1	2	1	1	2	1	1	1	1	1	2
	Z293	1	2	2	2	2	1	1	2	1	1	2	2	2	1	2	2
	Z294	1	2	1	2	1	1	2	2	1	1	1	1	1	2	2	2
	Z295	1	2	1	2	1	1	2	1	2	2	1	2	2	2	2	1
	Z296	2	1	1	2	2	2	2	1	1	1	2	2	2	2	2	2
	Z297	1	2	1	1	2	1	1	2	1	1	1	2	2	2	2	2
	Z300	2	1	2	1	2	1	2	2	2	1	1	2	2	1	2	2
	Z302	2	1	2	2	1	1	2	1	2	1	1	1	1	1	2	2
	Z303	2	2	2	2	2	1	2	1	2	2	1	2	2	1	2	2
	Z86	1 - 1	2 - 2	1 - 1	2 - 2	1 - 1	1 - 1	2 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1-2	1-1	2-2	1-2
	Z98	1 - 1	1 - 1	1 - 1	1 - 2	1 - 2	1 - 1	2 - 2	1 - 1	1 - 2	1 - 1	1 - 2	2 - 2	2-2	1-1	2-2	2-2
	Z139	1 - 2	2 - 2	1 - 2	1 - 2	2 - 2	1 - 1	2 - 2	2 - 2	1 - 1	1 - 1	1 - 1	2 - 2	2-2	1-1	2-2	1-2
	Z144	2 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 1	1 - 2	1 - 2	1 - 1	1 - 2	1 - 1	1 - 2	1-2	1-1	2-2	1-2
	Z153	1 - 2	2 - 2	1 - 1	1 - 1	1 - 2	1 - 1	2 - 2	2 - 2	2 - 2	1 - 2	1 - 1	1 - 2	1-2	1-1	1-2	2-2
	Z166	1 - 2	1 - 2	1 - 2	1 - 2	2 - 2	2 - 2	1 - 1	1 - 1	1 - 1	1 - 1	1 - 2	1 - 2	1-2	1-1	1-2	2-2
	Z209	2 - 2	1 - 2	1 - 2	1 - 2	1 - 2	2 - 2	2 - 2	1 - 1	1 - 2	1 - 2	1 - 1	1 - 2	1-2	1-1	2-2	2-2
	Z220	2 - 2	2 - 2	1 - 1	1 - 1	1 - 1	1 - 1	1 - 2	1 - 2	1 - 1	1 - 1	1 - 2	2 - 2	2-2	1-1	2-2	2-2
	Z256	1 - 1	1 - 1	1 - 1	1 - 2	1 - 1	1 - 2	2 - 2	2 - 2	1 - 2	2 - 2	1 - 1	1 - 1	1-1	1-1	2-2	2-2
	Z288	1 - 2	2 - 2	1 - 2	1 - 2	1 - 2	1 - 1	2 - 2	1 - 2	1 - 1	1 - 1	1 - 2	1 - 1	1-1	1-1	1-2	2-2

Table S3 X-chromosomal markers analysed in this study and their physical and genetic locations.

Marker	Physical Location (bps)	Genetic location (cM)
DXS10148	9,238,978	19.947
DXS10135	9,306,342	20.141
DXS8378	9,330,273	20.338
MID2612	10,234,839	24.181
MID3712	12,572,196	26.168
MID357	12,912,862	27.347
MID356	12,918,049	27.354
MID3703	13,711,300	30.347
MID3774	13,809,001	30.551
MID3692	19,516,253	37.059
MID3716	24,235,114	47.236
MID3690	28,984,077	49.881
MID3719	29,040,939	49.932
MID2089	29,157,973	50.059
MID2692	38,262,701	66.796
MID3701	45,539,202	80.142
MID198	47,680,387	84.174
DXS7132	64,655,336	90.753
DXS10079	66,715,903	90.820
DXS10074	66,977,187	90.835
MID1736	68,733,480	94.286
MID3730	88,009,690	102.120
MID1511	93,392,006	106.269
MID3740	97,906,547	111.498
MID3732	98,331,816	111.887
MID3727	99,165,489	112.735
MID3754	116,901,988	128.970
MID3722	118,156,158	130.403
MID1361	118,748,515	131.967
MID243	122,370,415	137.891
MID2637	124,135,529	141.717
MID111	127,958,385	145.077
MID3736	130,975,547	148.021
MID3753	131,760,173	148.230
DXS10103	133,418,989	149.607
HPRTB	133,615,516	150.024
DXS10101	133,654,515	150.010
MID1839	135,695,920	152.417
MID3760	137,369,795	153.122
MID329	147,393,784	179.333
DXS10146	149,584,306	184.645
DXS10134	149,650,120	184.847
DXS7423	149,710,955	185.060
MID2652	154,561,961	195.989

Physical location according to UCSC Genome Browser on Human Feb. 2009 GRCh37/hg19; Genetic location according to Rutgers map interpolator

Table S4 F_{ST} obtained for 8 X-STRs used in the MDS analysis.

	Miranda	Aliste	Bajo-Duero	Benavente	Campos-Pan	Sanabria	Sayago	Portugal (pt)	Portugal (po)	Galicia	Italy	Hungary	Poland	Finland
Miranda	0.000													
Aliste	0.002	0.000												
Bajo-Duero	0.007	0.002	0.000											
Benavente	0.006	0.004	0.004	0.000										
Campos-Pan	0.004	0.000	0.003	0.006	0.000									
Sanabria	0.013	0.005	0.001	0.000	0.007	0.000								
Sayago	0.015	0.001	0.001	0.014	0.000	0.007	0.000							
Portugal (pt)	0.006	0.004	0.001	0.000	0.004	0.001	0.005	0.000						
Portugal (po)	0.001	0.001	0.003	0.003	0.005	0.005	0.009	0.002	0.000					
Galicia	0.003	0.000	0.003	0.002	0.001	0.004	0.006	0.001	0.001	0.000				
Italy	0.004	0.002	0.004	0.005	0.001	0.002	0.002	0.001	0.001	0.000	0.000			
Hungary	0.003	0.003	0.004	0.003	0.002	0.005	0.007	0.002	0.003	0.001	0.000	0.000		
Poland	0.004	0.000	0.001	0.001	0.002	0.003	0.006	0.002	0.003	0.001	0.002	0.002	0.000	
Finland	0.006	0.000	0.004	0.004	0.002	0.008	0.005	0.005	0.004	0.002	0.003	0.003	0.001	0.000
Somalia	0.017	0.015	0.023	0.008	0.022	0.010	0.030	0.014	0.012	0.015	0.014	0.014	0.013	0.017
Algeria	0.003	0.003	0.001	0.002	0.003	0.003	0.007	0.002	0.003	0.002	0.002	0.003	0.002	0.005
Ghana	0.026	0.029	0.033	0.023	0.029	0.022	0.042	0.027	0.023	0.029	0.026	0.023	0.026	0.030
Ar. (Morocco)	0.004	0.014	0.007	0.006	0.012	0.007	0.019	0.004	0.003	0.008	0.004	0.004	0.007	0.011
Be. (Morocco)	0.006	0.003	0.011	0.005	0.012	0.001	0.012	0.002	0.005	0.002	0.004	0.004	0.007	0.010
Sh. (Morocco)	0.008	0.010	0.008	0.001	0.012	0.000	0.016	0.003	0.004	0.006	0.007	0.007	0.007	0.011
Ivory Coast	0.022	0.025	0.026	0.018	0.026	0.014	0.031	0.020	0.016	0.023	0.022	0.020	0.020	0.026
African US	0.019	0.023	0.022	0.013	0.021	0.013	0.030	0.017	0.015	0.019	0.018	0.016	0.017	0.021
Asian US	0.027	0.014	0.030	0.020	0.018	0.022	0.024	0.026	0.026	0.020	0.022	0.024	0.019	0.016
Caucasian US	0.003	0.003	0.006	0.001	0.004	0.005	0.011	0.003	0.002	0.001	0.003	0.000	0.001	0.003
Hispanic US	0.010	0.002	0.007	0.004	0.003	0.003	0.005	0.006	0.008	0.004	0.005	0.005	0.004	0.002
Germany	0.004	0.001	0.002	0.002	0.003	0.006	0.007	0.002	0.002	-0.000	0.001	0.000	0.001	0.002
China	0.029	0.018	0.034	0.018	0.019	0.018	0.027	0.024	0.027	0.022	0.021	0.024	0.021	0.018

	Somalia	Algeria	Ghana	Ar. (Morocco)	Be. (Morocco)	Sh. (Morocco)	Ivory Coast	African US	Asian US	Caucasian US	Hispanic US	Germany	China
Miranda													
Aliste													
Bajo-Duero													
Benavente													
Campos-Pan													
Sanabria													
Sayago													
Portugal (pt)													
Portugal (po)													
Galicia													
Italy													
Hungary													
Poland													
Finland													
Somalia	0.000												
Algeria	0.014	0.000											
Ghana	0.012	0.026	0.000										
Ar. (Morocco)	0.013	0.002	0.017	0.000									
Be. (Morocco)	0.012	0.005	0.023	0.004	0.000								
Sh. (Morocco)	0.009	0.002	0.019	0.001	0.006	0.000							
Ivory Coast	0.011	0.018	0.003	0.014	0.021	0.012	0.000						
African US	0.007	0.016	0.002	0.012	0.018	0.009	0.002	0.000					
Asian US	0.030	0.025	0.048	0.042	0.029	0.032	0.047	0.039	0.000				
Caucasian US	0.013	0.004	0.024	0.004	0.002	0.008	0.020	0.016	0.025	0.000			
Hispanic US	0.015	0.006	0.024	0.014	0.008	0.011	0.023	0.016	0.010	0.005	0.000		
Germany	0.015	0.003	0.027	0.006	0.006	0.007	0.021	0.017	0.023	0.001	0.006	0.000	
China	0.028	0.026	0.044	0.040	0.023	0.033	0.046	0.036	0.003	0.022	0.011	0.025	0.000